WEBVTT

NOTE duration:"01:01:40"

NOTE recognizability:0.931

NOTE language:en-us

NOTE Confidence: 0.92954665

00:00:00.000 --> 00:00:03.465 It's really an honor and pleasure to

NOTE Confidence: 0.92954665

00:00:03.465 --> 00:00:05.786 introduce to speak at Pathology Grand Rounds.

NOTE Confidence: 0.92954665

00:00:05.786 --> 00:00:08.400 Doctor Jerry Chippic, Professor of

NOTE Confidence: 0.92954665

00:00:08.400 --> 00:00:10.320 Oncological Sciences and Dermatology,

NOTE Confidence: 0.92954665

00:00:10.320 --> 00:00:12.320 Associate Director of Basic Science

NOTE Confidence: 0.92954665

00:00:12.440 --> 00:00:15.120 Shared Research Sources Director

NOTE Confidence: 0.92954665

00:00:15.120 --> 00:00:17.024 of Cell Biology at the I Can

NOTE Confidence: 0.92954665

00:00:17.024 --> 00:00:18.684 School of Medicine, Mount Sinai.

NOTE Confidence: 0.92954665

00:00:18.684 --> 00:00:21.638 Doctor Chippic completed his PhD at Case

NOTE Confidence: 0.92954665

00:00:21.638 --> 00:00:24.065 Western Reserve with David Daniel Poor

NOTE Confidence: 0.92954665

00:00:24.065 --> 00:00:27.319 working on cell death and TGF Beta Singling.

NOTE Confidence: 0.92954665

00:00:27.320 --> 00:00:28.840 He then completed postdoctoral

NOTE Confidence: 0.92954665

00:00:28.840 --> 00:00:30.360 work with Doug Greene,

NOTE Confidence: 0.92954665

00:00:30.360 --> 00:00:32.640 where he taught Doug most of

NOTE Confidence: 0.92954665

00:00:32.640 --> 00:00:35.079 what Doug knows about cell death.

NOTE Confidence: 0.92954665

00:00:35.080 --> 00:00:37.796 While there, he made seminal contributions to

NOTE Confidence: 0.92954665

00:00:37.796 --> 00:00:40.704 our understanding of P53 induced cell death.

NOTE Confidence: 0.92954665

00:00:40.704 --> 00:00:43.320 Puma's mechanism of action involvement of

NOTE Confidence: 0.92954665

00:00:43.387 --> 00:00:46.069 single lipids with backs back activation

NOTE Confidence: 0.92954665

00:00:46.070 --> 00:00:48.066 then started his own lab at Mount Sinai,

NOTE Confidence: 0.92954665

00:00:48.070 --> 00:00:50.002 where he continued to work on

NOTE Confidence: 0.92954665

00:00:50.002 --> 00:00:51.290 how mitochondrial morphology and

NOTE Confidence: 0.92954665

00:00:51.349 --> 00:00:52.909 function influence cell death,

NOTE Confidence: 0.92954665

00:00:52.910 --> 00:00:53.990 rass transformation,

NOTE Confidence: 0.92954665

00:00:53.990 --> 00:00:56.150 and map kinase activity,

NOTE Confidence: 0.92954665

00:00:56.150 --> 00:00:58.706 particularly within the context of Melanoma.

NOTE Confidence: 0.92954665

00:00:58.710 --> 00:01:00.910 It's also expanded our technical

NOTE Confidence: 0.92954665

00:01:00.910 --> 00:01:03.160 repertoire with personal favorites

NOTE Confidence: 0.92954665

00:01:03.160 --> 00:01:06.585 like sparkle and flambe assays,

NOTE Confidence: 0.92954665

00:01:06.590 --> 00:01:08.410 particularly inspiring those the way

NOTE Confidence: 0.92954665

00:01:08.410 --> 00:01:10.358 that Jerry serves as a mentor and

NOTE Confidence: 0.92954665

00:01:10.358 --> 00:01:12.430 champion of diversity within science.

NOTE Confidence: 0.92954665

00:01:12.430 --> 00:01:14.506 He sits on multiple DEI initiatives,

NOTE Confidence: 0.92954665

00:01:14.506 --> 00:01:16.396 mentored over 40 scientists directly

NOTE Confidence: 0.92954665

00:01:16.396 --> 00:01:18.501 within the lab ranging from high

NOTE Confidence: 0.92954665

00:01:18.501 --> 00:01:20.319 school students all the way up,

NOTE Confidence: 0.92954665

00:01:20.320 --> 00:01:21.940 including multiple first generation

NOTE Confidence: 0.92954665

00:01:21.940 --> 00:01:23.965 degree holders that he's mentored

NOTE Confidence: 0.92954665

00:01:23.965 --> 00:01:25.798 from technicians into Graduate

NOTE Confidence: 0.94080853

00:01:25.800 --> 00:01:27.920 School and studies. His

NOTE Confidence: 0.94080853

00:01:27.920 --> 00:01:29.204 mentorship and collegiality

NOTE Confidence: 0.94080853

00:01:29.204 --> 00:01:30.916 extends beyond Mount Sinai,

NOTE Confidence: 0.94080853

00:01:30.920 --> 00:01:32.600 and I've been very fortunate to get

NOTE Confidence: 0.94080853

00:01:32.600 --> 00:01:34.152 to know him at multiple meetings

NOTE Confidence: 0.94080853

00:01:34.152 --> 00:01:35.972 over the past 15 years and see

NOTE Confidence: 0.94080853

00:01:36.028 --> 00:01:37.558 him really is this insightful,

NOTE Confidence: 0.94080853

00:01:37.560 --> 00:01:39.940 humorous and caring colleague.

NOTE Confidence: 0.94080853

00:01:39.940 --> 00:01:42.036 I've always appreciated very

NOTE Confidence: 0.94080853

00:01:42.036 --> 00:01:43.848 much learning from him,

NOTE Confidence: 0.94080853

00:01:43.850 --> 00:01:46.328 both about the way he uses precision

NOTE Confidence: 0.94080853

00:01:46.328 --> 00:01:48.559 within his science and also the

NOTE Confidence: 0.94080853

00:01:48.559 --> 00:01:50.394 artistry of both his experimental

NOTE Confidence: 0.94080853

00:01:50.394 --> 00:01:52.289 design and his presentation,

NOTE Confidence: 0.94080853

00:01:52.290 --> 00:01:54.474 which I'm sure we'll all be able to

NOTE Confidence: 0.94080853

00:01:54.474 --> 00:01:56.437 enjoy and appreciate today as he

NOTE Confidence: 0.94080853

00:01:56.437 --> 00:01:58.471 speaks to us on the mitochondrial

NOTE Confidence: 0.94080853

00:01:58.539 --> 00:02:01.106 control of Melano Melanoma Genesis.

NOTE Confidence: 0.94080853

00:02:01.106 --> 00:02:03.410 Got it out.

NOTE Confidence: 0.94080853

00:02:03.410 --> 00:02:03.730 Please.

NOTE Confidence: 0.92801014

00:02:07.970 --> 00:02:10.338 All right. Well, First off, I'd like to

NOTE Confidence: 0.92801014

00:02:10.338 --> 00:02:12.860 extend my warmest appreciation to you, Sam.

NOTE Confidence: 0.92801014

00:02:12.860 --> 00:02:16.265 It's always lovely to come and visit friends

NOTE Confidence: 0.92801014

00:02:16.265 --> 00:02:18.620 and explore some of the science that we do.

NOTE Confidence: 0.92801014

00:02:18.620 --> 00:02:22.420 And I also want to say thank you to

NOTE Confidence: 0.92801014

00:02:22.420 --> 00:02:23.860 everyone in the audience for joining

NOTE Confidence: 0.92801014

00:02:23.906 --> 00:02:25.400 me this afternoon and everyone who

NOTE Confidence: 0.92801014

00:02:25.400 --> 00:02:26.940 joined me for dinner last night.

NOTE Confidence: 0.92801014

00:02:26.940 --> 00:02:29.340 I had a really lovely time.

NOTE Confidence: 0.92801014

00:02:29.340 --> 00:02:31.520 It's an honor to speak in in front of such

NOTE Confidence: 0.92801014

00:02:31.577 --> 00:02:33.617 an esteemed faculty and in member in front

NOTE Confidence: 0.92801014

00:02:33.617 --> 00:02:35.739 of the members of the Melanoma spore.

NOTE Confidence: 0.92801014

00:02:35.740 --> 00:02:39.538 So I'm excited to share our biology with you.

NOTE Confidence: 0.92801014

00:02:39.540 --> 00:02:42.225 My laboratory investigates how the

NOTE Confidence: 0.92801014

00:02:42.225 --> 00:02:44.822 mitochondrial network impacts upon general

NOTE Confidence: 0.92801014

00:02:44.822 --> 00:02:47.916 biology and we often position our studies

NOTE Confidence: 0.92801014

00:02:47.916 --> 00:02:50.848 in the context of cancer mechanisms

NOTE Confidence: 0.92801014

00:02:50.848 --> 00:02:53.464 to explore cellular transformation,

NOTE Confidence: 0.92801014

00:02:53.464 --> 00:02:56.296 chemotherapeutic success and prognosis.

NOTE Confidence: 0.92801014

00:02:56.300 --> 00:02:59.252 So today, in the next 45 to 50 minutes,

NOTE Confidence: 0.92801014

00:02:59.260 --> 00:03:02.028 I hope to provide you with a broad

NOTE Confidence: 0.92801014

00:03:02.028 --> 00:03:04.100 perspective of one of my group's

NOTE Confidence: 0.92801014

00:03:04.100 --> 00:03:06.413 research interests that centers on how

NOTE Confidence: 0.92801014

00:03:06.413 --> 00:03:08.521 the mitochondrial network influences

NOTE Confidence: 0.92801014

00:03:08.521 --> 00:03:10.654 melanocyte biology and how this

NOTE Confidence: 0.92801014

00:03:10.654 --> 00:03:12.086 provides some unique perspectives

NOTE Confidence: 0.92801014

00:03:12.086 --> 00:03:14.300 into the process of Melanoma genesis

NOTE Confidence: 0.938319

00:03:19.140 --> 00:03:22.750 here. OK, good.

NOTE Confidence: 0.938319

00:03:22.750 --> 00:03:26.068 So this research program originated by

NOTE Confidence: 0.938319

00:03:26.068 --> 00:03:28.280 observing mitochondrial shape changes

NOTE Confidence: 0.938319

00:03:28.354 --> 00:03:30.970 in how these shape changes reveal

NOTE Confidence: 0.938319

00:03:30.970 --> 00:03:33.230 insights into the cellular state.

NOTE Confidence: 0.938319

00:03:33.230 --> 00:03:35.708 But if you Google mitochondrial shape,

NOTE Confidence: 0.938319

00:03:35.710 --> 00:03:36.918 you'll get hundreds of

NOTE Confidence: 0.938319

00:03:36.918 --> 00:03:37.824 images of mitochondria.

NOTE Confidence: 0.938319

00:03:37.830 --> 00:03:39.910 That suggests mitochondrial shape

NOTE Confidence: 0.938319

00:03:39.910 --> 00:03:41.990 is boring and uninteresting.

NOTE Confidence: 0.938319

00:03:41.990 --> 00:03:43.532 However, I think it's time that

NOTE Confidence: 0.938319

00:03:43.532 --> 00:03:45.229 we kind of abandon this notion,

NOTE Confidence: 0.938319

00:03:45.230 --> 00:03:47.360 and because if you observe

NOTE Confidence: 0.938319

00:03:47.360 --> 00:03:49.064 mitochondria in living cells,

NOTE Confidence: 0.938319

00:03:49.070 --> 00:03:50.750 you will see just the opposite,

NOTE Confidence: 0.938319

00:03:50.750 --> 00:03:53.310 phenotype, and that is mitochondria

NOTE Confidence: 0.938319

00:03:53.310 --> 00:03:55.358 are dynamic organelles which

NOTE Confidence: 0.938319

00:03:55.358 --> 00:03:57.455 present themselves as a multitude

NOTE Confidence: 0.938319

00:03:57.455 --> 00:04:00.290 of sizes and a multitude of shapes.

NOTE Confidence: 0.938319

00:04:00.290 --> 00:04:03.034 And in this video you're looking at a

NOTE Confidence: 0.938319

00:04:03.034 --> 00:04:05.209 primary mouse embryonic fibroblast.

NOTE Confidence: 0.938319

00:04:05.210 --> 00:04:07.638 Its mitochondria are labeled with YFP

NOTE Confidence: 0.938319

00:04:07.638 --> 00:04:10.422 in the matrix and about 5 minutes of

NOTE Confidence: 0.938319

00:04:10.422 --> 00:04:12.249 mitochondrial movement is condensed

NOTE Confidence: 0.938319

00:04:12.249 --> 00:04:14.129 into approximately 3 seconds.

NOTE Confidence: 0.938319

00:04:14.130 --> 00:04:16.244 And in this this is a beauty.

NOTE Confidence: 0.938319

00:04:16.250 --> 00:04:17.339 In this video,

NOTE Confidence: 0.938319

00:04:17.339 --> 00:04:19.154 it's a really beautiful example

NOTE Confidence: 0.938319

00:04:19.154 --> 00:04:21.989 of looking at both intra and

NOTE Confidence: 0.938319

00:04:21.989 --> 00:04:23.009 interorganellar communication,

NOTE Confidence: 0.938319

00:04:23.010 --> 00:04:24.674 where metabolites,

NOTE Confidence: 0.938319

00:04:24.674 --> 00:04:25.506 proteins,

NOTE Confidence: 0.938319

00:04:25.506 --> 00:04:29.362 lipids and DNA are shared to

NOTE Confidence: 0.938319

00:04:29.362 --> 00:04:31.006 preserve mitochondrial network

NOTE Confidence: 0.938319

00:04:31.006 --> 00:04:32.650 homogeneity and efficiency.

NOTE Confidence: 0.938319

00:04:32.650 --> 00:04:35.205 And starting just over a decade ago,

NOTE Confidence: 0.938319

00:04:35.210 --> 00:04:37.630 I became interested in understanding

NOTE Confidence: 0.938319

00:04:37.630 --> 00:04:39.566 if fundamental mitochondrial biology

NOTE Confidence: 0.938319

00:04:39.570 --> 00:04:42.060 related to the dynamic nature of

NOTE Confidence: 0.938319

00:04:42.060 --> 00:04:44.006 this network influence cancer

NOTE Confidence: 0.938319

00:04:44.006 --> 00:04:46.796 mechanisms and offered any clinically

NOTE Confidence: 0.938319

00:04:46.796 --> 00:04:49.050 relevant insights into cancer.

NOTE Confidence: 0.938319

00:04:49.050 --> 00:04:50.226 And as such,

NOTE Confidence: 0.938319

00:04:50.226 --> 00:04:52.186 we choose to explore mitochondrial

NOTE Confidence: 0.938319

00:04:52.186 --> 00:04:55.202 function in the context of MAP kinase

NOTE Confidence: 0.938319

00:04:55.202 --> 00:04:56.930 signaling because its mutations

NOTE Confidence: 0.938319

00:04:56.930 --> 00:04:59.858 capture a majority of cellular tumors

NOTE Confidence: 0.938319

00:04:59.858 --> 00:05:01.798 and it demonstrates significant

NOTE Confidence: 0.938319

00:05:01.798 --> 00:05:03.420 therapeutic opportunities with

NOTE Confidence: 0.938319

00:05:03.420 --> 00:05:06.245 the advent of targeted therapies.

NOTE Confidence: 0.938319

00:05:06.250 --> 00:05:07.339 In this pathway,

NOTE Confidence: 0.938319

00:05:07.339 --> 00:05:09.154 signaling is normally initiated at

NOTE Confidence: 0.938319

00:05:09.154 --> 00:05:11.318 the level of the surface of the

NOTE Confidence: 0.938319

00:05:11.318 --> 00:05:13.169 plasma membrane by via a series

NOTE Confidence: 0.938319

00:05:13.169 --> 00:05:15.211 of receptor tyrosine kinases that

NOTE Confidence: 0.938319

00:05:15.211 --> 00:05:17.437 respond to extracellular ligands like e.g.

NOTE Confidence: 0.938319

00:05:17.440 --> 00:05:19.750 F and the signal is transmitted to

NOTE Confidence: 0.938319

00:05:19.750 --> 00:05:22.230 the nucleus via a series of GGP aces

NOTE Confidence: 0.938319

00:05:22.230 --> 00:05:24.965 such as Ras or kinases such as B

NOTE Confidence: 0.938319

00:05:24.965 --> 00:05:27.045 ref that enable transcriptional programs

NOTE Confidence: 0.938319

00:05:27.045 --> 00:05:29.638 that support survival and proliferation.

NOTE Confidence: 0.938319

00:05:29.638 --> 00:05:30.224 Interestingly,

NOTE Confidence: 0.938319

00:05:30.224 --> 00:05:31.982 pathway mutations also

NOTE Confidence: 0.938319

00:05:31.982 --> 00:05:34.240 occur in multiple nodes,

NOTE Confidence: 0.938319

00:05:34.240 --> 00:05:34.954 for example,

NOTE Confidence: 0.938319

00:05:34.954 --> 00:05:37.810 the receptor or Ras or B ref that

NOTE Confidence: 0.938319

00:05:37.897 --> 00:05:40.013 allow for constitutive signaling

NOTE Confidence: 0.938319

00:05:40.013 --> 00:05:42.658 through this pathway in the

NOTE Confidence: 0.938319

00:05:42.658 --> 00:05:44.898 absence of receptor ligation.

NOTE Confidence: 0.938319

00:05:44.900 --> 00:05:46.820 This pathway has also received a lot of

NOTE Confidence: 0.938319

00:05:46.820 --> 00:05:48.460 attention from drug companies of course,

NOTE Confidence: 0.938319

00:05:48.460 --> 00:05:50.455 as they have developed these

NOTE Confidence: 0.938319

00:05:50.455 --> 00:05:52.051 numerous specific and promising

NOTE Confidence: 0.938319

00:05:52.051 --> 00:05:53.782 small molecules that target

NOTE Confidence: 0.938319

00:05:53.782 --> 00:05:55.897 distinct nodes within the pathway.

NOTE Confidence: 0.938319

00:05:55.900 --> 00:05:57.116 Now for this talk,

NOTE Confidence: 0.938319

00:05:57.116 --> 00:05:58.940 I'm going to focus primarily on

NOTE Confidence: 0.938319

00:05:59.007 --> 00:06:00.378 mitochondrial phenotypes that

NOTE Confidence: 0.938319

00:06:00.378 --> 00:06:02.663 result from oncogenic MAP kinase

NOTE Confidence: 0.938319

00:06:02.663 --> 00:06:05.098 signaling in the context of Melanoma.

NOTE Confidence: 0.938319

00:06:05.100 --> 00:06:06.740 And a little bit later on I'm going

NOTE Confidence: 0.938319

00:06:06.740 --> 00:06:08.904 to bring up a few of these inhibitors

NOTE Confidence: 0.938319

00:06:08.904 --> 00:06:10.420 just throughout the experimental design.

NOTE Confidence: 0.938319

00:06:10.420 --> 00:06:11.458 So just keep them in mind.

NOTE Confidence: 0.94174147

00:06:13.510 --> 00:06:15.109 So First off,

NOTE Confidence: 0.94174147

00:06:15.109 --> 00:06:17.394 Melanoma is a curious disease.

NOTE Confidence: 0.94174147

00:06:17.394 --> 00:06:19.104 Curious is always my word.

NOTE Confidence: 0.94174147

00:06:19.110 --> 00:06:20.430 I use it to describe everything.

NOTE Confidence: 0.94174147

00:06:20.430 --> 00:06:22.830 It can be good and bad.

NOTE Confidence: 0.94174147

00:06:22.830 --> 00:06:24.350 It accounts for, you know,

NOTE Confidence: 0.94174147

00:06:24.350 --> 00:06:26.108 only about 4% of skin cancers,

NOTE Confidence: 0.94174147

00:06:26.110 --> 00:06:29.190 but about 80% of skin cancer deaths

NOTE Confidence: 0.94174147

00:06:29.190 --> 00:06:31.776 incidence is increasing even in nations

NOTE Confidence: 0.94174147

00:06:31.776 --> 00:06:34.870 where Melanoma has been historically lower.

NOTE Confidence: 0.94174147

00:06:34.870 --> 00:06:36.382 I'm going to talk about maybe

NOTE Confidence: 0.94174147

00:06:36.382 --> 00:06:38.126 some of the reasons for this a

NOTE Confidence: 0.94174147

00:06:38.126 --> 00:06:39.494 little bit later in the talk.

NOTE Confidence: 0.94174147

00:06:39.500 --> 00:06:41.250 Secondly, I want to introduce the cell

NOTE Confidence: 0.94174147

00:06:41.250 --> 00:06:43.334 line or the cell of origin which is

NOTE Confidence: 0.94174147

00:06:43.334 --> 00:06:45.419 related to disease and it's the melanocyte.

NOTE Confidence: 0.94174147

00:06:45.420 --> 00:06:46.188 You know,

NOTE Confidence: 0.94174147

00:06:46.188 --> 00:06:48.242 cell type is really important

NOTE Confidence: 0.94174147

00:06:48.242 --> 00:06:50.046 when we consider mitochondrial

NOTE Confidence: 0.94174147

00:06:50.046 --> 00:06:51.912 function as melanocytes are derived

NOTE Confidence: 0.94174147

00:06:51.912 --> 00:06:54.030 from the neural tube and often

NOTE Confidence: 0.94174147

00:06:54.092 --> 00:06:55.924 straddle the interface between

NOTE Confidence: 0.94174147

00:06:55.924 --> 00:06:57.756 epithelial and neuronal phenotypes

NOTE Confidence: 0.94174147

00:06:57.756 --> 00:07:00.099 from the organelle perspective.

NOTE Confidence: 0.94174147

00:07:00.100 --> 00:07:02.368 And these cells are just normally

NOTE Confidence: 0.94174147

00:07:02.368 --> 00:07:04.760 kind of dotted along the skin.

NOTE Confidence: 0.94174147

00:07:04.760 --> 00:07:06.080 They synthesize melanin,

NOTE Confidence: 0.94174147

00:07:06.080 --> 00:07:08.152 and they transfer it in the form of

NOTE Confidence: 0.94174147

00:07:08.152 --> 00:07:10.303 these tiny parcels from the epidermal

NOTE Confidence: 0.94174147

00:07:10.303 --> 00:07:11.879 basal layer to keratinocytes,

NOTE Confidence: 0.94174147

00:07:11.880 --> 00:07:14.065 where melanin then is responsible

NOTE Confidence: 0.94174147

00:07:14.065 --> 00:07:16.250 for dissipating UV energy to

NOTE Confidence: 0.94174147

00:07:16.324 --> 00:07:18.439 prevent macromolecular damage.

NOTE Confidence: 0.94174147

00:07:18.440 --> 00:07:20.757 Here I'm showing a very simplified version,

NOTE Confidence: 0.94174147

00:07:20.760 --> 00:07:22.856 our process of melanomagenesis,

NOTE Confidence: 0.94174147

00:07:22.856 --> 00:07:25.476 which emphasizes a series of

NOTE Confidence: 0.94174147

00:07:25.476 --> 00:07:27.114 histopathological features associated

NOTE Confidence: 0.94174147

00:07:27.114 --> 00:07:29.709 with primary and invasive disease.

NOTE Confidence: 0.94174147

00:07:29.710 --> 00:07:32.165 But what happens between normal

NOTE Confidence: 0.94174147

00:07:32.165 --> 00:07:34.950 skin and primary Melanoma in C2?

NOTE Confidence: 0.94174147

00:07:34.950 --> 00:07:35.994 And, you know,

NOTE Confidence: 0.94174147

00:07:35.994 --> 00:07:38.082 the answer to this is often

NOTE Confidence: 0.94174147

00:07:38.082 --> 00:07:39.941 associated with benign skin lesions

NOTE Confidence: 0.94174147

00:07:39.941 --> 00:07:42.107 known as nevi or common moles,

NOTE Confidence: 0.94174147

00:07:42.110 --> 00:07:44.392 which are kind of minimally described as

NOTE Confidence: 0.94174147

00:07:44.392 --> 00:07:46.669 these clusters of senescent melanocytes.

NOTE Confidence: 0.94174147

00:07:46.670 --> 00:07:48.110 And if you look at your arms or legs,

NOTE Confidence: 0.94174147

00:07:48.110 --> 00:07:49.158 you find a mole.

NOTE Confidence: 0.94174147

00:07:49.158 --> 00:07:50.468 And more likely than that,

NOTE Confidence: 0.94174147

00:07:50.470 --> 00:07:51.610 there's a mutation there for

NOTE Confidence: 0.94174147

00:07:51.610 --> 00:07:53.541 B Rocky 600 E that drives the

NOTE Confidence: 0.94174147

00:07:53.541 --> 00:07:55.025 proliferation of those melanocytes.

NOTE Confidence: 0.9237536

00:07:57.570 --> 00:08:00.461 So in the laboratory, we can model

NOTE Confidence: 0.9237536

00:08:00.461 --> 00:08:03.113 Melanoma genesis by isolating primary

NOTE Confidence: 0.9237536

00:08:03.113 --> 00:08:05.769 melanocytes from healthy patients.

NOTE Confidence: 0.9237536

00:08:05.770 --> 00:08:07.954 And then we infect them with an

NOTE Confidence: 0.9237536

00:08:07.954 --> 00:08:09.956 UNCLE gene like B ref E600E or

NOTE Confidence: 0.9237536

00:08:09.956 --> 00:08:12.287 an UNCLE gene and an RNAI against

NOTE Confidence: 0.9237536

00:08:12.287 --> 00:08:14.890 a tumor suppressor such as P10.

NOTE Confidence: 0.9237536

00:08:14.890 --> 00:08:17.045 And this combination of treatments

NOTE Confidence: 0.9237536

00:08:17.045 --> 00:08:20.319 allows us to compare the biology of

NOTE Confidence: 0.9237536

00:08:20.319 --> 00:08:22.929 primary melanocytes to senescent cells,

NOTE Confidence: 0.9237536

00:08:22.930 --> 00:08:25.145 and how overriding senescence may

NOTE Confidence: 0.9237536

00:08:25.145 --> 00:08:28.130 eventually lead to a transform phenotype.

NOTE Confidence: 0.9237536

00:08:28.130 --> 00:08:30.170 And when you observe mitochondrial

NOTE Confidence: 0.9237536

00:08:30.170 --> 00:08:32.210 networks in these representative cells,

NOTE Confidence: 0.9237536

00:08:32.210 --> 00:08:35.150 you can easily determine that there are

NOTE Confidence: 0.9237536

00:08:35.150 --> 00:08:37.977 abundant changes as we transition from

NOTE Confidence: 0.9237536

00:08:37.977 --> 00:08:40.965 normal to senescent to transform phenotypes,

NOTE Confidence: 0.9237536

00:08:40.970 --> 00:08:43.278 which together represents multiple

NOTE Confidence: 0.9237536

00:08:43.278 --> 00:08:46.163 research programs within my group.

NOTE Confidence: 0.9237536

00:08:46.170 --> 00:08:49.046 And looking at these mitochondrial

NOTE Confidence: 0.9237536

00:08:49.046 --> 00:08:51.460 networks a little bit in more detail,

NOTE Confidence: 0.9237536

00:08:51.460 --> 00:08:53.616 now here you see this normal milanocyte,

NOTE Confidence: 0.9237536

00:08:53.620 --> 00:08:57.075 it has a beautiful normal

NOTE Confidence: 0.9237536

00:08:57.075 --> 00:08:58.457 mitochondrial architecture.

NOTE Confidence: 0.9237536

00:08:58.460 --> 00:08:59.986 And then when you introduce the raffin

NOTE Confidence: 0.9237536

00:08:59.986 --> 00:09:01.896 to them and you undergo the process

NOTE Confidence: 0.9237536

00:09:01.896 --> 00:09:03.100 of oncogene induced senescence,

NOTE Confidence: 0.9237536

00:09:03.100 --> 00:09:04.724 of course you see the cell getting

NOTE Confidence: 0.9237536

00:09:04.724 --> 00:09:05.940 larger because it's senescence.

NOTE Confidence: 0.9237536

00:09:05.940 --> 00:09:08.100 Everybody calls it the flat egg

NOTE Confidence: 0.9237536

00:09:08.100 --> 00:09:10.340 phenotype or the fried egg phenotype.

NOTE Confidence: 0.9237536

00:09:10.340 --> 00:09:12.804 But what you also notice is this marked

NOTE Confidence: 0.9237536

00:09:12.804 --> 00:09:15.019 expansion of the mitochondrial network.

NOTE Confidence: 0.9237536

00:09:15.020 --> 00:09:17.540 And here we're standing for HSP 60,

NOTE Confidence: 0.9237536

00:09:17.540 --> 00:09:20.030 which is a mitochondrial matrix marker.

NOTE Confidence: 0.9237536

00:09:20.030 --> 00:09:21.885 And you can see that there's also

NOTE Confidence: 0.9237536

00:09:21.885 --> 00:09:23.869 a lot greater intensity of H SP60.

NOTE Confidence: 0.9237536

00:09:23.870 --> 00:09:27.550 So we have more mitochondria in these cells.

NOTE Confidence: 0.9237536

00:09:27.550 --> 00:09:30.826 But why generate all these mitochondria

NOTE Confidence: 0.9237536

00:09:30.830 --> 00:09:33.548 if these cells are destined to

NOTE Confidence: 0.9237536

00:09:33.548 --> 00:09:34.907 never divide again?

NOTE Confidence: 0.9237536

00:09:34.910 --> 00:09:37.010 Why build 3 powerhouses in a

NOTE Confidence: 0.9237536

00:09:37.010 --> 00:09:38.893 community of homes that's never

NOTE Confidence: 0.9237536

00:09:38.893 --> 00:09:40.948 going to increase in population?

NOTE Confidence: 0.9237536

00:09:40.950 --> 00:09:44.387 And do these changes impact on metabolism

NOTE Confidence: 0.9237536

00:09:44.387 --> 00:09:47.638 signaling or the fate of these cells?

NOTE Confidence: 0.9237536

00:09:47.640 --> 00:09:48.876 And using this as a platform,

NOTE Confidence: 0.9237536

00:09:48.880 --> 00:09:50.818 we're going to investigate some of

NOTE Confidence: 0.9237536

00:09:50.818 --> 00:09:52.677 the roles of these mitochondrial

NOTE Confidence: 0.9237536

00:09:52.677 --> 00:09:54.677 changes in these processes.

NOTE Confidence: 0.9237536

00:09:54.680 --> 00:09:56.560 So to explore these questions,

NOTE Confidence: 0.9237536

00:09:56.560 --> 00:09:58.930 I already mentioned earlier that we

NOTE Confidence: 0.9237536

00:09:58.930 --> 00:10:01.448 model the early stages of Melanoma

NOTE Confidence: 0.9237536

00:10:01.448 --> 00:10:03.782 genesis and here you're just looking at

NOTE Confidence: 0.9237536

00:10:03.782 --> 00:10:05.828 some bright filled images of primary

NOTE Confidence: 0.9237536

00:10:05.828 --> 00:10:07.633 melanocytes and how they change

NOTE Confidence: 0.9237536

00:10:07.633 --> 00:10:09.794 morphology during Aqua gene induced in

NOTE Confidence: 0.9237536

00:10:09.794 --> 00:10:12.167 essence that's initiated by B raft signaling.

NOTE Confidence: 0.9237536

00:10:12.170 --> 00:10:14.210 And we see this really beautiful.

NOTE Confidence: 0.9237536

00:10:14.210 --> 00:10:16.338 You can see melanocytes are not the

NOTE Confidence: 0.9237536

00:10:16.338 --> 00:10:18.698 most attractive cells when in just the

NOTE Confidence: 0.9237536

00:10:18.698 --> 00:10:20.086 control conditions they're spindly.

NOTE Confidence: 0.9237536

00:10:20.090 --> 00:10:21.126 You look at them on the microscope,

NOTE Confidence: 0.9237536

00:10:21.130 --> 00:10:22.219 they're not gorgeous,

NOTE Confidence: 0.9237536

00:10:22.219 --> 00:10:24.034 but you induce opportunity to

NOTE Confidence: 0.9237536

00:10:24.034 --> 00:10:26.113 senescence and now a majority of

NOTE Confidence: 0.9237536

00:10:26.113 --> 00:10:27.405 them become really beautiful,

NOTE Confidence: 0.9237536

00:10:27.410 --> 00:10:29.090 amazing architectures and phenotypes.

NOTE Confidence: 0.9237536

00:10:29.090 --> 00:10:32.321 We can see that of course these cells

NOTE Confidence: 0.9237536

00:10:32.321 --> 00:10:34.732 express the B rat P600E Aqua gene.

NOTE Confidence: 0.9237536

00:10:34.732 --> 00:10:36.976 The majority of them have senescence

NOTE Confidence: 0.9237536

00:10:36.976 --> 00:10:39.050 associated beta galactocytase activity.

NOTE Confidence: 0.9237536

00:10:39.050 --> 00:10:42.050 We can detect P21P16 expression in

NOTE Confidence: 0.9237536

00:10:42.050 --> 00:10:44.210 these cells and they also secrete

NOTE Confidence: 0.9237536

00:10:44.210 --> 00:10:47.388 all of the classical markers of the

NOTE Confidence: 0.9237536

00:10:47.388 --> 00:10:49.224 senescence associated secretory phenotype.

NOTE Confidence: 0.9237536

00:10:49.230 --> 00:10:50.358 And here we're just measuring the

NOTE Confidence: 0.9237536

00:10:50.358 --> 00:10:51.110 RN A's from them,

NOTE Confidence: 0.9308397

00:10:53.990 --> 00:10:55.030 data I'm not showing you.

NOTE Confidence: 0.9308397

00:10:55.030 --> 00:10:56.864 Of course, we've also looked at Saps

NOTE Confidence: 0.9308397

00:10:56.864 --> 00:10:59.242 and we've also looked at a few other

NOTE Confidence: 0.9308397

00:10:59.242 --> 00:11:00.474 morphological features to confirm

NOTE Confidence: 0.9308397

00:11:00.474 --> 00:11:02.427 that this process is what we expect.

NOTE Confidence: 0.9308397

00:11:02.430 --> 00:11:05.104 So now let's investigate a little bit

NOTE Confidence: 0.9308397

00:11:05.104 --> 00:11:07.870 about how BR FP600 signaling actually

NOTE Confidence: 0.9308397

00:11:07.870 --> 00:11:10.370 alters mitochondrial biology to connect

NOTE Confidence: 0.9308397

00:11:10.370 --> 00:11:13.369 some of these questions I asked earlier.

NOTE Confidence: 0.9308397

00:11:13.370 --> 00:11:15.176 So we first measure in these

NOTE Confidence: 0.9308397

00:11:15.176 --> 00:11:16.809 situations E CAR and O CAR.

NOTE Confidence: 0.9308397

00:11:16.810 --> 00:11:18.976 This is looking at the extracellular

NOTE Confidence: 0.9308397

00:11:18.976 --> 00:11:20.868 acidification rate or the oxygen

NOTE Confidence: 0.9308397

00:11:20.868 --> 00:11:22.808 consumption rates in these cells.

NOTE Confidence: 0.9308397

00:11:22.810 --> 00:11:25.134 And we do this by using the

NOTE Confidence: 0.9308397

00:11:25.134 --> 00:11:26.130 Agilent Seahorse technology,

NOTE Confidence: 0.9308397

00:11:26.130 --> 00:11:28.489 where we do everything in real time.

NOTE Confidence: 0.9308397

00:11:28.490 --> 00:11:30.436 And measuring E CAR and O CAR

NOTE Confidence: 0.9308397

00:11:30.436 --> 00:11:33.688 allows us to look at glycolysis and

NOTE Confidence: 0.9308397

00:11:33.688 --> 00:11:35.449 mitochondrial respiration respectively.

NOTE Confidence: 0.9308397

00:11:35.450 --> 00:11:37.880 And here what you can see is when you

NOTE Confidence: 0.9308397

00:11:37.880 --> 00:11:39.528 introduce B ref into these cells,

NOTE Confidence: 0.9308397

00:11:39.530 --> 00:11:41.110 they're normally quite glycolytic.

NOTE Confidence: 0.9308397

00:11:41.110 --> 00:11:44.169 And when you introduce B ref into them,

NOTE Confidence: 0.9308397

00:11:44.170 --> 00:11:46.050 their glycolysis completely collapses

NOTE Confidence: 0.9308397

00:11:46.050 --> 00:11:48.870 and they have a coordinated increase

NOTE Confidence: 0.9308397

00:11:48.936 --> 00:11:50.968 in their mitochondrial function.

NOTE Confidence: 0.9308397

00:11:50.970 --> 00:11:53.345 They have a coordinated increase

NOTE Confidence: 0.9308397

00:11:53.345 --> 00:11:55.245 in their basal respiration,

NOTE Confidence: 0.9308397

00:11:55.250 --> 00:11:57.410 their maximal respiration and

NOTE Confidence: 0.9308397

00:11:57.410 --> 00:11:59.570 their spare respiratory capacity.

NOTE Confidence: 0.9308397

00:11:59.570 --> 00:12:01.658 And you can see this is a kind of

NOTE Confidence: 0.9308397

00:12:01.658 --> 00:12:02.902 amazing re metabolic programming

NOTE Confidence: 0.9308397

00:12:02.902 --> 00:12:05.121 of these cells when you put the

NOTE Confidence: 0.9308397

00:12:05.183 --> 00:12:06.765 B ref on the gene into them.

NOTE Confidence: 0.9397675

00:12:08.980 --> 00:12:11.619 So digging a bit deeper into the

NOTE Confidence: 0.9397675

00:12:11.620 --> 00:12:15.388 bioenergetics of these cells to explain

NOTE Confidence: 0.9397675

00:12:15.388 --> 00:12:17.900 the enhanced mitochondrial respiration,

NOTE Confidence: 0.9397675

00:12:17.900 --> 00:12:20.078 we look to see who's actually

NOTE Confidence: 0.9397675

00:12:20.078 --> 00:12:21.530 responsible for providing electrons

NOTE Confidence: 0.9397675

00:12:21.591 --> 00:12:23.376 to the electron transport chain.

NOTE Confidence: 0.9397675

00:12:23.380 --> 00:12:27.403 If we remember NADH supplies to complex 1F,

NOTE Confidence: 0.9397675

00:12:27.403 --> 00:12:29.524 ADH supplies to complex 2 and that's

NOTE Confidence: 0.9397675

00:12:29.524 --> 00:12:31.390 how you stimulate respiration to

NOTE Confidence: 0.9397675

00:12:31.390 --> 00:12:34.176 drive proton pumping to create what we

NOTE Confidence: 0.9397675

00:12:34.241 --> 00:12:36.980 call mitochondrial delta psi or the

NOTE Confidence: 0.9397675

00:12:36.980 --> 00:12:38.920 bioenergetic differences between the

NOTE Confidence: 0.9397675

00:12:38.920 --> 00:12:41.768 intermembrane and the rest of the cell.

NOTE Confidence: 0.9397675

00:12:41.770 --> 00:12:42.806 And if you look at this here,

NOTE Confidence: 0.9397675

00:12:42.810 --> 00:12:45.050 you'll see that both Complex

NOTE Confidence: 0.9397675

00:12:45.050 --> 00:12:46.842 One is perfectly fine,

NOTE Confidence: 0.9397675

00:12:46.850 --> 00:12:48.405 Complex 2 is perfectly fine

NOTE Confidence: 0.9397675

00:12:48.405 --> 00:12:49.649 in the control cells.

NOTE Confidence: 0.9397675

00:12:49.650 --> 00:12:51.906 But in both situations when you

NOTE Confidence: 0.9397675

00:12:51.906 --> 00:12:54.169 introduce the rapid 600 E oncogen,

NOTE Confidence: 0.9397675

00:12:54.170 --> 00:12:56.966 both Complex one and Complex 2

NOTE Confidence: 0.9397675

00:12:56.966 --> 00:12:59.650 markedly increase in their activity.

NOTE Confidence: 0.9397675

00:12:59.650 --> 00:13:03.545 And so the way that we study

NOTE Confidence: 0.9397675

00:13:03.545 --> 00:13:05.313 this is normally driven by State

NOTE Confidence: 0.9397675

00:13:05.313 --> 00:13:06.627 3 respiration measurements,

NOTE Confidence: 0.9397675

00:13:06.630 --> 00:13:08.828 which basically just means if you remember

NOTE Confidence: 0.9397675

00:13:08.828 --> 00:13:10.910 the concepts of respiratory control,

NOTE Confidence: 0.9397675

00:13:10.910 --> 00:13:14.004 whenever the level of ADP is markedly

NOTE Confidence: 0.9397675

00:13:14.004 --> 00:13:16.910 higher compared to the levels of a TP,

NOTE Confidence: 0.9397675

00:13:16.910 --> 00:13:18.142 you can stimulate respiration

NOTE Confidence: 0.9397675

00:13:18.142 --> 00:13:19.066 within these cells.

NOTE Confidence: 0.9397675

00:13:19.070 --> 00:13:21.436 And this is what this is basically

NOTE Confidence: 0.9397675

00:13:21.436 --> 00:13:23.630 looking at State 3 driven respiration

NOTE Confidence: 0.9397675

00:13:23.630 --> 00:13:26.227 to either Complex one or Complex 2.

NOTE Confidence: 0.9397675

00:13:26.230 --> 00:13:27.734 So B,

NOTE Confidence: 0.9397675

00:13:27.734 --> 00:13:31.494 roughly 600 E markedly renovates

NOTE Confidence: 0.9397675

00:13:31.500 --> 00:13:33.246 Melanicites mitochondrial network

NOTE Confidence: 0.9397675

00:13:33.246 --> 00:13:35.574 to become more productive.

NOTE Confidence: 0.9397675

00:13:35.580 --> 00:13:38.649 And we can see here are some just some

NOTE Confidence: 0.9397675

00:13:38.649 --> 00:13:41.540 nice examples of this basal respiration

NOTE Confidence: 0.9397675

00:13:41.540 --> 00:13:45.162 changes By about eightfold the spare

NOTE Confidence: 0.9397675

00:13:45.162 --> 00:13:48.217 capacity increases by about fourfold

NOTE Confidence: 0.9397675

00:13:48.220 --> 00:13:51.611 and it tells us that wild B ref.

NOTE Confidence: 0.9397675

00:13:51.611 --> 00:13:54.233 E 600 E is building more

NOTE Confidence: 0.9397675

00:13:54.233 --> 00:13:56.218 powerhouses within the cell.

NOTE Confidence: 0.9397675

00:13:56.220 --> 00:13:58.488 It's not in a manner that necessarily

NOTE Confidence: 0.9397675

00:13:58.488 --> 00:14:00.340 allows them to be efficient,

NOTE Confidence: 0.9397675

00:14:00.340 --> 00:14:02.548 because if you measure the consequences

NOTE Confidence: 0.9397675

00:14:02.548 --> 00:14:04.020 of these mitochondrial expansions,

NOTE Confidence: 0.9397675

00:14:04.020 --> 00:14:06.785 you'll see something that's very

NOTE Confidence: 0.9397675

00:14:06.785 --> 00:14:09.035 curious and that it's easy to

NOTE Confidence: 0.9397675

00:14:09.035 --> 00:14:10.730 determine that cellular fitness was

NOTE Confidence: 0.9397675

00:14:10.798 --> 00:14:13.063 not considered when these melanocytes

NOTE Confidence: 0.9397675

00:14:13.063 --> 00:14:14.875 were generating mitochondria when

NOTE Confidence: 0.9397675

00:14:14.875 --> 00:14:16.938 they encounter B RAF signaling.

NOTE Confidence: 0.9397675

00:14:16.940 --> 00:14:18.440 And here we're just looking at

NOTE Confidence: 0.9397675

00:14:18.440 --> 00:14:20.260 a few assays to quantify this.

NOTE Confidence: 0.9397675

00:14:20.260 --> 00:14:22.460 In A and B, we're looking at mitosox.

NOTE Confidence: 0.9397675

00:14:22.460 --> 00:14:24.448 This is kind of a very standard

NOTE Confidence: 0.9397675

00:14:24.448 --> 00:14:26.351 assay looking for reactive oxygen

NOTE Confidence: 0.9397675

00:14:26.351 --> 00:14:28.771 species generation and it basically

NOTE Confidence: 0.9397675

00:14:28.771 --> 00:14:30.679 represents when electrons aren't

NOTE Confidence: 0.9397675

00:14:30.679 --> 00:14:32.510 properly moving between iron,

NOTE Confidence: 0.9397675

00:14:32.510 --> 00:14:34.258 sulfur cluster groups within

NOTE Confidence: 0.9397675

00:14:34.258 --> 00:14:35.569 individual complexes within

NOTE Confidence: 0.9397675

00:14:35.569 --> 00:14:37.270 the electron transport chain,

NOTE Confidence: 0.9397675

00:14:37.270 --> 00:14:39.700 or when these electrons are not

NOTE Confidence: 0.9397675

00:14:39.700 --> 00:14:41.320 flowing efficiently between the

NOTE Confidence: 0.9397675

00:14:41.392 --> 00:14:43.707 complexes that drive proton pumping.

NOTE Confidence: 0.94767356

00:14:45.830 --> 00:14:48.798 We also can see that mitochondrial Ross

NOTE Confidence: 0.94767356

00:14:48.798 --> 00:14:51.697 leads to protein oxidation versus the

NOTE Confidence: 0.94767356

00:14:51.697 --> 00:14:54.438 generation or due to the generation

NOTE Confidence: 0.94767356

00:14:54.438 --> 00:14:57.000 of reactive oxygen species and radical

NOTE Confidence: 0.94767356

00:14:57.077 --> 00:14:59.597 species such as superoxide and hydroxyls

NOTE Confidence: 0.94767356

00:14:59.597 --> 00:15:02.822 and also these non radical species such

NOTE Confidence: 0.94767356

00:15:02.822 --> 00:15:05.762 as hydrogen peroxide and cichlid oxygens.

NOTE Confidence: 0.94767356

00:15:05.770 --> 00:15:09.051 All of these target prolines, arginines,

NOTE Confidence: 0.94767356

00:15:09.051 --> 00:15:11.656 lysines, threonine residues within your

NOTE Confidence: 0.94767356

00:15:11.656 --> 00:15:14.370 proteins and compromised protein function,

NOTE Confidence: 0.94767356

00:15:14.370 --> 00:15:15.729 assembly and quality.

NOTE Confidence: 0.94767356

00:15:15.729 --> 00:15:17.088 And then finally,

NOTE Confidence: 0.94767356

00:15:17.090 --> 00:15:20.048 we also measured melon D aldehyde,

NOTE Confidence: 0.94767356

00:15:20.050 --> 00:15:23.128 which is the final step of

NOTE Confidence: 0.94767356

00:15:23.130 --> 00:15:24.606 polyunsaturated fatty acid peroxidation.

NOTE Confidence: 0.94767356

00:15:24.606 --> 00:15:27.164 And you can see it's also increased

NOTE Confidence: 0.94767356

00:15:27.164 --> 00:15:28.886 in the presence of B raft.

NOTE Confidence: 0.94767356

00:15:28.890 --> 00:15:31.242 So what this basically tells us is

NOTE Confidence: 0.94767356

00:15:31.242 --> 00:15:33.385 increasing B raft V600 signaling

NOTE Confidence: 0.94767356

00:15:33.385 --> 00:15:35.509 stimulates Mart mitochondrial Ross

NOTE Confidence: 0.94767356

00:15:35.509 --> 00:15:37.633 production protein oxidation and

NOTE Confidence: 0.94767356

00:15:37.699 --> 00:15:40.464 also oxidation of the of the membrane

NOTE Confidence: 0.94767356

00:15:40.464 --> 00:15:41.943 compartments within the cell.

NOTE Confidence: 0.94767356

00:15:41.943 --> 00:15:43.701 And what's important to note in

NOTE Confidence: 0.94767356

00:15:43.701 --> 00:15:45.549 this situation is we're not looking

NOTE Confidence: 0.94767356

00:15:45.549 --> 00:15:46.765 at very small changes,

NOTE Confidence: 0.94767356

00:15:46.770 --> 00:15:48.306 we're actually looking at

NOTE Confidence: 0.94767356

00:15:48.306 --> 00:15:49.458 quite significant changes.

NOTE Confidence: 0.94767356

00:15:49.460 --> 00:15:50.580 If you notice here,

NOTE Confidence: 0.94767356

00:15:50.580 --> 00:15:53.092 the control in these cells are always FCCP.

NOTE Confidence: 0.94767356

00:15:53.092 --> 00:15:54.820 And this is a,

NOTE Confidence: 0.94767356

00:15:54.820 --> 00:15:55.468 you know,

NOTE Confidence: 0.94767356

00:15:55.468 --> 00:15:56.764 it's the classical uncoupler

NOTE Confidence: 0.94767356

00:15:56.764 --> 00:15:57.736 of mitochondrial biology.

NOTE Confidence: 0.94767356

00:15:57.740 --> 00:16:00.700 It basically is in the cytosol of cells,

NOTE Confidence: 0.94767356

00:16:00.700 --> 00:16:02.908 it takes protons and drags them

NOTE Confidence: 0.94767356

00:16:02.908 --> 00:16:04.380 inside the mitochondrial matrix.

NOTE Confidence: 0.94767356

00:16:04.380 --> 00:16:06.212 And when that happens,

NOTE Confidence: 0.94767356

00:16:06.212 --> 00:16:08.502 the mitochondrial respond by increasing

NOTE Confidence: 0.94767356

00:16:08.502 --> 00:16:10.972 their ability to pump or to move

NOTE Confidence: 0.94767356

00:16:10.972 --> 00:16:13.104 electrons and to pump protons to

NOTE Confidence: 0.94767356

00:16:13.104 --> 00:16:15.778 compensate for that change in Delta psi.

NOTE Confidence: 0.94767356

00:16:15.780 --> 00:16:18.720 FCCP is the classical maximal ability

NOTE Confidence: 0.94767356

00:16:18.720 --> 00:16:20.755 for mitochondria to do something

NOTE Confidence: 0.94767356

00:16:20.755 --> 00:16:24.007 which you can see with Ross protein

NOTE Confidence: 0.94767356

00:16:24.007 --> 00:16:26.279 oxidation and lipid peroxidation.

NOTE Confidence: 0.94767356

00:16:26.280 --> 00:16:27.224 B ref.

NOTE Confidence: 0.94767356

00:16:27.224 --> 00:16:29.584 E600E signaling almost equals the

NOTE Confidence: 0.94767356

00:16:29.584 --> 00:16:31.532 same level as FCCP treatment,

NOTE Confidence: 0.94767356

00:16:31.532 --> 00:16:33.394 so it's a significant amount of damage

NOTE Confidence: 0.94767356

00:16:33.394 --> 00:16:34.800 that's happening to these cells.

NOTE Confidence: 0.9325133

00:16:37.040 --> 00:16:39.360 So after identifying these phenotypes,

NOTE Confidence: 0.9325133

00:16:39.360 --> 00:16:42.440 we see marked mitochondrial expansion,

NOTE Confidence: 0.9325133

00:16:42.440 --> 00:16:46.310 we see enhanced respiration, we see

NOTE Confidence: 0.9325133

00:16:46.310 --> 00:16:48.245 consequential macromolecular oxidation.

NOTE Confidence: 0.9325133

00:16:48.250 --> 00:16:51.010 We next questioned if the mitochondrial

NOTE Confidence: 0.9325133

00:16:51.010 --> 00:16:54.170 network itself started to signal for help

NOTE Confidence: 0.9325133

00:16:54.170 --> 00:16:57.166 to assist with their own quality control.

NOTE Confidence: 0.9325133

00:16:57.170 --> 00:16:58.900 And this is basically driven

NOTE Confidence: 0.9325133

00:16:58.900 --> 00:17:01.090 here because you can see again,

NOTE Confidence: 0.9325133

00:17:01.090 --> 00:17:02.530 and I showed you these earlier,

NOTE Confidence: 0.9325133

00:17:02.530 --> 00:17:04.810 these mitochondria are getting much larger.

NOTE Confidence: 0.9325133

00:17:04.810 --> 00:17:06.230 And what drives this expansion

NOTE Confidence: 0.9325133

00:17:06.230 --> 00:17:07.366 of the mitochondrial network?

NOTE Confidence: 0.9325133

00:17:07.370 --> 00:17:10.278 Well, normally it's proteins like PGC 1A,

NOTE Confidence: 0.9325133

00:17:10.278 --> 00:17:12.616 which is a key protein involved in

NOTE Confidence: 0.9325133

00:17:12.616 --> 00:17:15.565 the transcription of metabolism genes

NOTE Confidence: 0.9325133

00:17:15.565 --> 00:17:18.105 and also mitochondrial bioenergetics

NOTE Confidence: 0.9325133

00:17:18.110 --> 00:17:20.063 and mitochondrial Biogenesis.

NOTE Confidence: 0.9325133

00:17:20.063 --> 00:17:22.915 But we also see TPM,

NOTE Confidence: 0.9325133

00:17:22.915 --> 00:17:24.630 which is one of the major transcription

NOTE Confidence: 0.9325133

00:17:24.630 --> 00:17:26.694 factors of the mitochondrial network that's

NOTE Confidence: 0.9325133

00:17:26.694 --> 00:17:28.594 responsible for driving mitochondrial RNA

NOTE Confidence: 0.9325133

00:17:28.594 --> 00:17:30.270 polymerase to the mitochondrial genome.

NOTE Confidence: 0.9325133

00:17:30.270 --> 00:17:31.670 And we also look at another marker,

NOTE Confidence: 0.9325133

00:17:31.670 --> 00:17:34.729 Tom 20, which is just a classical

NOTE Confidence: 0.9325133

00:17:34.729 --> 00:17:36.510 marker of mitochondrial mass.

NOTE Confidence: 0.9325133

00:17:36.510 --> 00:17:38.532 So all of these markers of

NOTE Confidence: 0.9325133

00:17:38.532 --> 00:17:40.110 mitochondrial function are going up.

NOTE Confidence: 0.9325133

00:17:40.110 --> 00:17:42.530 We see there's increased mitochondrial

NOTE Confidence: 0.9325133

00:17:42.530 --> 00:17:44.466 function and marked inefficiency.

NOTE Confidence: 0.9325133

00:17:44.470 --> 00:17:46.854 So it wasn't a surprise to us when

NOTE Confidence: 0.9325133

00:17:46.854 --> 00:17:49.000 we started to screen for stress

NOTE Confidence: 0.9325133

00:17:49.000 --> 00:17:51.464 within the network that these

NOTE Confidence: 0.9325133

00:17:51.464 --> 00:17:52.974 signaling pathways were turned on.

NOTE Confidence: 0.9325133

00:17:52.980 --> 00:17:56.424 And in particular we looked at a TF5ATF4

NOTE Confidence: 0.9325133

00:17:56.424 --> 00:17:59.656 and D TI-3 which is also called Chopped.

NOTE Confidence: 0.9325133

00:17:59.660 --> 00:18:02.740 And these are the three transcription factors

NOTE Confidence: 0.9325133

00:18:02.740 --> 00:18:05.369 that drive the canonical mitochondrial

NOTE Confidence: 0.9325133

00:18:05.369 --> 00:18:08.459 unfolded protein response in cells.

NOTE Confidence: 0.9325133

00:18:08.460 --> 00:18:10.479 And these transcription

NOTE Confidence: 0.9325133

00:18:10.479 --> 00:18:12.498 factors are activated.

NOTE Confidence: 0.9325133

00:18:12.500 --> 00:18:14.404 Here we're looking at the level of RNA,

NOTE Confidence: 0.9325133

00:18:14.404 --> 00:18:16.516 but they're also each one of them have

NOTE Confidence: 0.9325133

00:18:16.516 --> 00:18:18.775 a cohort of transcriptional targets that

NOTE Confidence: 0.9325133

00:18:18.775 --> 00:18:21.503 they induce to then restore the quality

NOTE Confidence: 0.9325133

00:18:21.503 --> 00:18:24.058 of the mitochondrial network back to normal.

NOTE Confidence: 0.9325133

00:18:24.060 --> 00:18:26.460 And all of these classical transcription

NOTE Confidence: 0.9325133

00:18:26.460 --> 00:18:28.980 targets of the pathway are also activated.

NOTE Confidence: 0.9325133

00:18:28.980 --> 00:18:31.296 And you can see that indeed.

NOTE Confidence: 0.9325133

00:18:31.300 --> 00:18:35.348 So let's take a little bit deeper into the

NOTE Confidence: 0.9325133

00:18:35.348 --> 00:18:37.300 mitochondrial unfolded protein response.

NOTE Confidence: 0.9325133

00:18:37.300 --> 00:18:39.799 You know the the MiTo UPR activates

NOTE Confidence: 0.9325133

00:18:39.799 --> 00:18:41.769 following a host of stressors

NOTE Confidence: 0.9325133

00:18:41.770 --> 00:18:43.278 commonly in the literature.

NOTE Confidence: 0.9325133

00:18:43.278 --> 00:18:45.163 It can be metabolic distress,

NOTE Confidence: 0.9325133

00:18:45.170 --> 00:18:47.204 it can be overexpressing mutant proteins

NOTE Confidence: 0.9325133

00:18:47.204 --> 00:18:49.708 in the matrix or the inner membrane

NOTE Confidence: 0.9325133

00:18:49.708 --> 00:18:51.886 space of mitochondria that are just,

NOTE Confidence: 0.9325133

00:18:51.890 --> 00:18:53.102 you know,

NOTE Confidence: 0.9325133

00:18:53.102 --> 00:18:55.526 overexpressed in recombinant mutants.

NOTE Confidence: 0.9325133

00:18:55.530 --> 00:18:58.090 But it can also be induced by infecting

NOTE Confidence: 0.9325133

00:18:58.090 --> 00:19:00.371 cells and a lot of the literature

NOTE Confidence: 0.9325133

00:19:00.371 --> 00:19:02.530 looks at infections in C elegans.

NOTE Confidence: 0.9325133

00:19:02.530 --> 00:19:05.410 Or if you infect helis cells

NOTE Confidence: 0.9325133

00:19:05.410 --> 00:19:06.370 with pseudomonas,

NOTE Confidence: 0.9325133

00:19:06.370 --> 00:19:09.424 you can activate this pathway and

NOTE Confidence: 0.9325133

00:19:09.424 --> 00:19:12.292 what it does is basically exists as

NOTE Confidence: 0.9325133

00:19:12.292 --> 00:19:14.312 a beautiful example of mitonuclear

NOTE Confidence: 0.9325133

00:19:14.312 --> 00:19:14.716 communication.

NOTE Confidence: 0.9325133

00:19:14.720 --> 00:19:16.160 Those are the three transcription

NOTE Confidence: 0.9325133

00:19:16.160 --> 00:19:17.600 factors that are responsible for

NOTE Confidence: 0.9325133

00:19:17.651 --> 00:19:18.916 the ones I mentioned earlier,

NOTE Confidence: 0.9325133

00:19:18.920 --> 00:19:20.900 and they induce A transcriptional

NOTE Confidence: 0.9325133

00:19:20.900 --> 00:19:22.880 and epigenetic program to restore

NOTE Confidence: 0.9325133

00:19:22.947 --> 00:19:24.957 the quality of these mitochondria.

NOTE Confidence: 0.9325133

00:19:24.960 --> 00:19:27.016 And the way they do this is by

NOTE Confidence: 0.9325133

00:19:27.016 --> 00:19:28.479 increasing protein folding capacity,

NOTE Confidence: 0.9325133

00:19:28.480 --> 00:19:30.900 Ross protection and protease

NOTE Confidence: 0.9325133

00:19:30.900 --> 00:19:33.320 activation within the network.

NOTE Confidence: 0.9325133

00:19:33.320 --> 00:19:36.002 And it's just a really beautiful

NOTE Confidence: 0.9325133

00:19:36.002 --> 00:19:38.339 example of how mitochondria and

NOTE Confidence: 0.9325133

00:19:38.339 --> 00:19:40.699 the nucleus can actually interact.

NOTE Confidence: 0.9325133

00:19:40.700 --> 00:19:44.298 And the reason why is the miter.

NOTE Confidence: 0.9325133

00:19:44.300 --> 00:19:47.858 UPR depends primarily on a TF5,

NOTE Confidence: 0.9325133

00:19:47.860 --> 00:19:50.555 and this is a basic lucine zipper

NOTE Confidence: 0.9325133

00:19:50.555 --> 00:19:52.318 transcription factor containing an

NOTE Confidence: 0.9325133

00:19:52.318 --> 00:19:53.953 n-terminal mitochondrial targeting

NOTE Confidence: 0.9325133

00:19:53.953 --> 00:19:57.084 sequence and A/C terminal nuclear

NOTE Confidence: 0.9325133

00:19:57.084 --> 00:19:58.376 localization signal.

NOTE Confidence: 0.9325133

00:19:58.380 --> 00:20:01.320 And upon normal conditions ATF5IS

NOTE Confidence: 0.9325133

00:20:01.320 --> 00:20:02.496 constitutively expressed.

NOTE Confidence: 0.9325133

00:20:02.500 --> 00:20:03.920 And because the mitochondrial

NOTE Confidence: 0.9325133

00:20:03.920 --> 00:20:05.340 targeting sequence is there,

NOTE Confidence: 0.93575937

00:20:05.340 --> 00:20:06.995 it's always read by the

NOTE Confidence: 0.93575937

00:20:06.995 --> 00:20:07.657 mitochondrial network,

NOTE Confidence: 0.93575937

00:20:07.660 --> 00:20:08.863 imported and degraded.

NOTE Confidence: 0.93575937

00:20:08.863 --> 00:20:10.868 And when there's an aberration

NOTE Confidence: 0.93575937

00:20:10.868 --> 00:20:12.250 in mitochondrial function,

NOTE Confidence: 0.93575937

00:20:12.250 --> 00:20:14.578 because delta psi starts to decrease

NOTE Confidence: 0.93575937

00:20:14.578 --> 00:20:16.130 because efficiency is changing,

NOTE Confidence: 0.93575937

00:20:16.130 --> 00:20:17.814 proton pumping is changing,

NOTE Confidence: 0.93575937

00:20:17.814 --> 00:20:19.919 the nuclear localization signal starts

NOTE Confidence: 0.93575937

00:20:19.919 --> 00:20:21.903 to get detected more frequently

NOTE Confidence: 0.93575937

00:20:21.903 --> 00:20:24.562 within the cytosol and you shift from

NOTE Confidence: 0.93575937

00:20:24.562 --> 00:20:26.362 mitochondrial import to nuclear import

NOTE Confidence: 0.93575937

00:20:26.362 --> 00:20:28.610 to then drive a transcriptional program.

NOTE Confidence: 0.93575937

00:20:28.610 --> 00:20:32.362 So it's a nice way where one protein can

NOTE Confidence: 0.93575937

00:20:32.362 --> 00:20:34.767 communicate the quality of mitochondria.

NOTE Confidence: 0.93575937

00:20:34.770 --> 00:20:37.050 So in a broader context,

NOTE Confidence: 0.93575937

00:20:37.050 --> 00:20:42.450 ATF5 normally functions with TOP and a TF4,

NOTE Confidence: 0.93575937

00:20:42.450 --> 00:20:45.380 and it's important to note this because

NOTE Confidence: 0.93575937

00:20:45.380 --> 00:20:46.950 almost all the literature suggests

NOTE Confidence: 0.93575937

00:20:47.002 --> 00:20:48.377 that they always cooperate together

NOTE Confidence: 0.93575937

00:20:48.377 --> 00:20:50.319 and one of the things we're going

NOTE Confidence: 0.93575937

00:20:50.319 --> 00:20:52.111 to see here is a unique dependency

NOTE Confidence: 0.93575937

00:20:52.111 --> 00:20:54.970 almost exclusively on a TF5 biology.

NOTE Confidence: 0.92992723

00:20:57.890 --> 00:21:00.330 I also need to suggest that or to

NOTE Confidence: 0.92992723

00:21:00.330 --> 00:21:02.530 say there's a tremendous literature

NOTE Confidence: 0.92992723

00:21:02.530 --> 00:21:05.370 on a TF5 already in cancer,

NOTE Confidence: 0.92992723

00:21:05.370 --> 00:21:07.368 but the biology of a TF5I

NOTE Confidence: 0.92992723

00:21:07.368 --> 00:21:09.158 think needs to be reevaluated.

NOTE Confidence: 0.92992723

00:21:09.160 --> 00:21:11.200 Because once we recognize it

NOTE Confidence: 0.92992723

00:21:11.200 --> 00:21:12.832 as the major transcription

NOTE Confidence: 0.92992723

00:21:12.832 --> 00:21:14.957 factor for the MiTo UPR pathway,

NOTE Confidence: 0.92992723

00:21:14.960 --> 00:21:17.080 I think we have to kind of look at the

NOTE Confidence: 0.92992723

00:21:17.144 --> 00:21:19.160 literature and say what makes sense,

NOTE Confidence: 0.92992723

00:21:19.160 --> 00:21:21.440 what doesn't make sense from the

NOTE Confidence: 0.92992723

00:21:21.440 --> 00:21:22.960 mitochondrial perspective and reintegrate

NOTE Confidence: 0.92992723

00:21:23.020 --> 00:21:24.590 this literature and reexamine some

NOTE Confidence: 0.92992723

00:21:24.590 --> 00:21:26.715 of the phenotypes that are generated

NOTE Confidence: 0.92992723

00:21:26.715 --> 00:21:30.600 when we regulate ATF5 in both normal

NOTE Confidence: 0.92992723

00:21:30.600 --> 00:21:34.200 cells and also transformed states.

NOTE Confidence: 0.92992723

00:21:34.200 --> 00:21:36.513 So this is just showing a quick example of

NOTE Confidence: 0.92992723

00:21:36.513 --> 00:21:38.956 what ATF 5 looks like if you stay in for it.

NOTE Confidence: 0.92992723

00:21:38.960 --> 00:21:40.838 Some of the antibodies are terrible.

NOTE Confidence: 0.92992723

00:21:40.840 --> 00:21:42.718 And there's also another issue and

NOTE Confidence: 0.92992723

00:21:42.718 --> 00:21:44.917 that is you'll see if you put your

NOTE Confidence: 0.92992723

00:21:44.920 --> 00:21:47.080 FP600E when it's spelled wrong.

NOTE Confidence: 0.92992723

00:21:47.080 --> 00:21:48.262 And how do you figure that

NOTE Confidence: 0.92992723

00:21:48.262 --> 00:21:49.320 out when you're at Yale?

NOTE Confidence: 0.92992723

00:21:49.320 --> 00:21:50.304 That I can't spell.

NOTE Confidence: 0.92992723

00:21:50.304 --> 00:21:52.998 All right, this is what I learned at Yale.

NOTE Confidence: 0.92992723

00:21:53.000 --> 00:21:54.332 You'll see little speckles of ATF5

NOTE Confidence: 0.92992723

00:21:54.332 --> 00:21:55.760 and the image there is terrible.

NOTE Confidence: 0.92992723

00:21:55.760 --> 00:21:56.960 I guess everybody always says

NOTE Confidence: 0.92992723

00:21:56.960 --> 00:21:58.160 that it looks beautiful here.

NOTE Confidence: 0.92992723

00:21:58.160 --> 00:21:59.852 ATF 5 accumulates in the nucleus

NOTE Confidence: 0.92992723

00:21:59.852 --> 00:22:01.520 when you add an Oncogen.

NOTE Confidence: 0.92992723

00:22:01.520 --> 00:22:02.468 And unfortunately,

NOTE Confidence: 0.92992723

00:22:02.468 --> 00:22:04.838 the antibodies always pick up

NOTE Confidence: 0.92992723

00:22:04.838 --> 00:22:06.870 the degraded peptides as well.

NOTE Confidence: 0.92992723

00:22:06.870 --> 00:22:08.262 So the cytosis kind of flooded

NOTE Confidence: 0.92992723

00:22:08.262 --> 00:22:09.862 with these A TF5 peptides that

NOTE Confidence: 0.92992723

00:22:09.862 --> 00:22:11.397 are degraded by the mitochondrial

NOTE Confidence: 0.92992723

00:22:11.397 --> 00:22:13.309 network and it also picks up that.

NOTE Confidence: 0.92992723

00:22:13.310 --> 00:22:14.590 So there's always the cytosol,

NOTE Confidence: 0.92992723

00:22:14.590 --> 00:22:16.790 that contribution that you see,

NOTE Confidence: 0.92992723

00:22:16.790 --> 00:22:18.614 but all of the cells that

NOTE Confidence: 0.92992723

00:22:18.614 --> 00:22:20.190 you infect with B ref,

NOTE Confidence: 0.92992723

00:22:20.190 --> 00:22:22.406 We've also done work with Ras, H Ras,

NOTE Confidence: 0.92992723

00:22:22.406 --> 00:22:24.422 N Ras all activate the same pathway.

NOTE Confidence: 0.92992723

00:22:24.430 --> 00:22:26.320 But for this talk I'm just

NOTE Confidence: 0.92992723

00:22:26.320 --> 00:22:28.070 going to talk about B ref.

NOTE Confidence: 0.92992723

00:22:28.070 --> 00:22:30.290 So what connects these pathways?

NOTE Confidence: 0.92992723

00:22:30.290 --> 00:22:32.530 How do you go from B rough to a TF5?

NOTE Confidence: 0.92992723

00:22:32.530 --> 00:22:35.071 And what makes a TF5 unique and

NOTE Confidence: 0.92992723

00:22:35.071 --> 00:22:37.050 interesting from this perspective?

NOTE Confidence: 0.92992723

00:22:37.050 --> 00:22:40.004 Well the A TF5 dependent minor UPR

NOTE Confidence: 0.92992723

00:22:40.004 --> 00:22:42.263 literature doesn't implicate any post

NOTE Confidence: 0.92992723

00:22:42.263 --> 00:22:44.111 translational modifications within this

NOTE Confidence: 0.92992723

00:22:44.111 --> 00:22:47.169 pathway to allow for organelle repair.

NOTE Confidence: 0.92992723

00:22:47.170 --> 00:22:48.946 But there are descriptions of P300

NOTE Confidence: 0.92992723

00:22:48.946 --> 00:22:51.045 or CBP which is a transcriptional

NOTE Confidence: 0.92992723

00:22:51.045 --> 00:22:54.027 coactivator that acts in part through the

NOTE Confidence: 0.92992723

00:22:54.027 --> 00:22:56.562 accetalation of his stones and non his

NOTE Confidence: 0.92992723

00:22:56.562 --> 00:22:58.730 stone substrates that satellites ATF5.

NOTE Confidence: 0.92992723

00:22:58.730 --> 00:23:02.710 And there are roles in the serum responses,

NOTE Confidence: 0.92992723

00:23:02.710 --> 00:23:05.190 there are roles in infection,

NOTE Confidence: 0.92992723

00:23:05.190 --> 00:23:07.430 there are roles in adepogenesis

NOTE Confidence: 0.92992723

00:23:07.430 --> 00:23:09.670 and longevity and C elegans.

NOTE Confidence: 0.92992723

00:23:09.670 --> 00:23:11.590 And these observations let us

NOTE Confidence: 0.92992723

00:23:11.590 --> 00:23:13.510 to investigate if onpogenic map

NOTE Confidence: 0.92992723

00:23:13.582 --> 00:23:15.717 kinase signaling also could change

NOTE Confidence: 0.92992723

00:23:15.717 --> 00:23:17.425 a TF5 settlation patterns.

NOTE Confidence: 0.92992723

00:23:17.430 --> 00:23:20.270 So what we did was we took linocytes,

NOTE Confidence: 0.92992723

00:23:20.270 --> 00:23:23.990 infected them with B RP600E purified

NOTE Confidence: 0.92992723

00:23:23.990 --> 00:23:26.087 ATF5 and you can see that we see a

NOTE Confidence: 0.92992723

00:23:26.087 --> 00:23:27.999 nice acetylation bands on a TF5.

NOTE Confidence: 0.92992723

00:23:28.000 --> 00:23:29.908 And if you do this in the presence of

NOTE Confidence: 0.92992723

00:23:29.908 --> 00:23:32.037 Matt kinase inhibitors or P300 inhibitors,

NOTE Confidence: 0.92992723

00:23:32.040 --> 00:23:33.432 you lose the acetylation.

NOTE Confidence: 0.92992723

00:23:33.432 --> 00:23:35.520 We then take that purified protein

NOTE Confidence: 0.92992723

00:23:35.584 --> 00:23:37.904 and then subject it to mass spec to

NOTE Confidence: 0.92992723

00:23:37.904 --> 00:23:39.522 identify where the acetylation takes

NOTE Confidence: 0.92992723

00:23:39.522 --> 00:23:42.440 place and it takes place on lysine 29.

NOTE Confidence: 0.92992723

00:23:42.440 --> 00:23:45.558 And what's curious is the acetylation

NOTE Confidence: 0.92992723

00:23:45.558 --> 00:23:47.511 is not there for the Matt kinase

NOTE Confidence: 0.92992723

00:23:47.511 --> 00:23:49.242 inhibitor or P300 inhibitor and if

NOTE Confidence: 0.92992723

00:23:49.242 --> 00:23:50.637 you also stress the mitochondrial

NOTE Confidence: 0.9294755

00:23:50.694 --> 00:23:51.779 network, it's not there.

NOTE Confidence: 0.9294755

00:23:51.779 --> 00:23:54.168 This is the FCCP result, and also,

NOTE Confidence: 0.9294755

00:23:54.168 --> 00:23:56.278 if you activate the mitochondria,

NOTE Confidence: 0.9294755

00:23:56.280 --> 00:23:57.808 unfolded protein response from

NOTE Confidence: 0.9294755

00:23:57.808 --> 00:23:59.336 within the mitochondria by

NOTE Confidence: 0.9294755

00:23:59.336 --> 00:24:01.120 inhibiting one of the chaperones.

NOTE Confidence: 0.9294755

00:24:01.120 --> 00:24:03.200 That's the small molecule GTPP.

NOTE Confidence: 0.9294755

00:24:03.200 --> 00:24:05.558 It also is not there suggesting that

NOTE Confidence: 0.9294755

00:24:05.558 --> 00:24:08.192 this is a specific modification to

NOTE Confidence: 0.9294755

00:24:08.192 --> 00:24:10.566 this pathway that's dependent upon

NOTE Confidence: 0.9294755

00:24:10.566 --> 00:24:13.410 B ref and not necessarily conserved

NOTE Confidence: 0.9294755

00:24:13.410 --> 00:24:15.560 throughout all MiTo UPR responses.

NOTE Confidence: 0.9294755

00:24:15.560 --> 00:24:17.600 We can make mutants of this

NOTE Confidence: 0.9294755

00:24:17.600 --> 00:24:19.644 a TF5 wild type K29 Q,

NOTE Confidence: 0.9294755

00:24:19.644 --> 00:24:21.354 which is the acetylation mimic,

NOTE Confidence: 0.9294755

00:24:21.360 --> 00:24:23.850 or K29R which is an acetylation

NOTE Confidence: 0.9294755

00:24:23.850 --> 00:24:25.775 null mutant of a TF5.

NOTE Confidence: 0.9294755

00:24:25.775 --> 00:24:27.936 You can put it into melanocytes,

NOTE Confidence: 0.9294755

00:24:27.936 --> 00:24:29.616 you can stimulate them with

NOTE Confidence: 0.9294755

00:24:29.616 --> 00:24:30.960 a MiTo UPR inducer,

NOTE Confidence: 0.9294755

00:24:30.960 --> 00:24:34.720 and all of them will stabilize as normal.

NOTE Confidence: 0.9294755

00:24:34.720 --> 00:24:37.198 And what's curious is looking at the

NOTE Confidence: 0.9294755

00:24:37.198 --> 00:24:40.329 impact of a TF5 on actually senescence.

NOTE Confidence: 0.9294755

00:24:40.330 --> 00:24:42.808 So here we're looking at melanocytes,

NOTE Confidence: 0.9294755

00:24:42.810 --> 00:24:44.930 we infect them with B ref with 600

NOTE Confidence: 0.9294755

00:24:44.930 --> 00:24:46.849 E looking at 3 days and 21 days.

NOTE Confidence: 0.9294755

00:24:46.850 --> 00:24:49.458 You can see you get a nice time

NOTE Confidence: 0.9294755

00:24:49.458 --> 00:24:51.449 dependent increase in beta galactosidase

NOTE Confidence: 0.9294755

00:24:51.449 --> 00:24:53.569 and senescence in these cells.

NOTE Confidence: 0.9294755

00:24:53.570 --> 00:24:55.778 If you silence ATF5,

NOTE Confidence: 0.9294755

00:24:55.778 --> 00:24:59.090 all the cells almost immediately sines.

NOTE Confidence: 0.9294755

00:24:59.090 --> 00:25:01.604 In the presence of B ref, they're fine.

NOTE Confidence: 0.9294755

00:25:01.604 --> 00:25:04.338 In the absence of of B ref, they will.

NOTE Confidence: 0.9294755

00:25:04.338 --> 00:25:05.730 They will persist.

NOTE Confidence: 0.9294755

00:25:05.730 --> 00:25:07.260 We introduced a combination within

NOTE Confidence: 0.9294755

00:25:07.260 --> 00:25:09.204 three days the majority of the

NOTE Confidence: 0.9294755

00:25:09.204 --> 00:25:10.648 population is already senescent,

NOTE Confidence: 0.9294755

00:25:10.650 --> 00:25:12.910 suggesting that the MiTo UPR

NOTE Confidence: 0.9294755

00:25:12.910 --> 00:25:15.170 pathway actually puts the brakes

NOTE Confidence: 0.9294755

00:25:15.244 --> 00:25:17.168 on the senescence pathway.

NOTE Confidence: 0.9294755

00:25:17.170 --> 00:25:18.970 And then if you reconstitute with wild type,

NOTE Confidence: 0.9294755

00:25:18.970 --> 00:25:20.670 you see a similar situation

NOTE Confidence: 0.9294755

00:25:20.670 --> 00:25:22.370 to the wild type state.

NOTE Confidence: 0.9294755

00:25:22.370 --> 00:25:24.350 And what's curious is if you

NOTE Confidence: 0.9294755

00:25:24.350 --> 00:25:26.130 put in the acetalation mimic,

NOTE Confidence: 0.9294755

00:25:26.130 --> 00:25:29.496 this is the form of a TF5 that looks

NOTE Confidence: 0.9294755

00:25:29.496 --> 00:25:31.407 constitutively acetalated by B ref.

NOTE Confidence: 0.9294755

00:25:31.410 --> 00:25:33.370 It completely blocks B ref.

NOTE Confidence: 0.9294755

00:25:33.370 --> 00:25:35.920 Induced on the induced senescence

NOTE Confidence: 0.9294755

00:25:35.920 --> 00:25:38.400 and if you prevent acetylation,

NOTE Confidence: 0.9294755

00:25:38.400 --> 00:25:40.310 all the cells again become

NOTE Confidence: 0.9294755

00:25:40.310 --> 00:25:41.537 senescent immediately suggesting

NOTE Confidence: 0.9294755

00:25:41.537 --> 00:25:43.822 that this pathway really uniquely

NOTE Confidence: 0.9294755

00:25:43.822 --> 00:25:46.127 integrates BRF signaling with Uncle

NOTE Confidence: 0.9294755

00:25:46.127 --> 00:25:49.200 Jeannie Duce senescence via ATF5.

NOTE Confidence: 0.9294755

00:25:49.200 --> 00:25:51.174 And these are just some of the

NOTE Confidence: 0.9294755

00:25:51.174 --> 00:25:52.020 different transcriptional programs

NOTE Confidence: 0.9294755

00:25:52.068 --> 00:25:53.526 that are regulated by these mutants

NOTE Confidence: 0.9294755

00:25:53.526 --> 00:25:55.239 looking at the minor UPR perspective.

NOTE Confidence: 0.9294755

00:25:55.240 --> 00:25:56.612 And these are some of the outcomes

NOTE Confidence: 0.9294755

00:25:56.612 --> 00:25:59.798 of these cells, just some images.

NOTE Confidence: 0.9294755

00:25:59.800 --> 00:26:02.240 So I mentioned earlier that

NOTE Confidence: 0.9294755

00:26:02.240 --> 00:26:05.530 there's a unite a unique MiTo UPR

NOTE Confidence: 0.9294755

00:26:05.530 --> 00:26:07.248 comparing B ref A600E signaling

NOTE Confidence: 0.9294755

00:26:07.248 --> 00:26:09.120 to this small molecule that the

NOTE Confidence: 0.9294755

00:26:09.181 --> 00:26:11.011 majority of the literature uses

NOTE Confidence: 0.9294755

00:26:11.011 --> 00:26:12.789 to activate this pathway GTPP.

NOTE Confidence: 0.9294755

00:26:12.789 --> 00:26:15.261 And here you can see B ref A600

NOTE Confidence: 0.9294755

00:26:15.261 --> 00:26:17.295 deactivates the SASS P The normal MiTo

NOTE Confidence: 0.9294755

00:26:17.295 --> 00:26:19.793 UPR pathway or at least the small

NOTE Confidence: 0.9294755

00:26:19.793 --> 00:26:21.557 molecule induced pathway doesn't.

NOTE Confidence: 0.9294755

00:26:21.560 --> 00:26:22.757 There's differential regulation

NOTE Confidence: 0.9294755

00:26:22.757 --> 00:26:25.151 of the minor UPR targets to

NOTE Confidence: 0.9294755

00:26:25.151 --> 00:26:27.159 correct mitochondrial dysfunction.

NOTE Confidence: 0.9294755

00:26:27.160 --> 00:26:28.892 This differential regulation of

NOTE Confidence: 0.9294755

00:26:28.892 --> 00:26:31.057 mitochondrial included genes When you

NOTE Confidence: 0.9294755

00:26:31.057 --> 00:26:33.276 do this and just one way to summarize

NOTE Confidence: 0.9294755

00:26:33.276 --> 00:26:35.516 this as well is just looking at T fam.

NOTE Confidence: 0.9294755

00:26:35.520 --> 00:26:36.760 T fam is not induced.

NOTE Confidence: 0.9294755

00:26:36.760 --> 00:26:39.215 This is the transcription factor

NOTE Confidence: 0.9294755

00:26:39.215 --> 00:26:41.179 again that's responsible for

NOTE Confidence: 0.9294755

00:26:41.179 --> 00:26:42.720 creating the mRNA transcripts

NOTE Confidence: 0.9294755

00:26:42.720 --> 00:26:44.240 from the mitochondrial genome.

NOTE Confidence: 0.9294755

00:26:44.240 --> 00:26:46.892 Also not induced from the canonical

NOTE Confidence: 0.9294755

00:26:46.892 --> 00:26:49.400 MiTo UPR always specific to B ref.

NOTE Confidence: 0.9294755

00:26:49.400 --> 00:26:51.308 And then finally we can study

NOTE Confidence: 0.9294755

00:26:51.308 --> 00:26:53.120 some of these metabolic changes.

NOTE Confidence: 0.9294755

00:26:53.120 --> 00:26:54.674 You may remember I said when you

NOTE Confidence: 0.9294755

00:26:54.674 --> 00:26:56.399 put B ref into a Melano site,

NOTE Confidence: 0.9090125

00:26:56.400 --> 00:26:58.400 they go from becoming glycolytic

NOTE Confidence: 0.9090125

00:26:58.400 --> 00:27:00.199 to mitochondrial and that is

NOTE Confidence: 0.9090125

00:27:00.199 --> 00:27:01.864 conserved in these data here.

NOTE Confidence: 0.9090125

00:27:01.870 --> 00:27:03.403 But when you treat with GTPP

NOTE Confidence: 0.9090125

00:27:03.403 --> 00:27:05.068 they do just the opposite.

NOTE Confidence: 0.9090125

00:27:05.070 --> 00:27:06.834 They collapse their mitochondrial

NOTE Confidence: 0.9090125

00:27:06.834 --> 00:27:09.039 function and they increase their

NOTE Confidence: 0.9090125

00:27:09.039 --> 00:27:10.773 glycolytic activity just again showing

NOTE Confidence: 0.9090125

00:27:10.773 --> 00:27:13.014 that this is a very unique UPR.

NOTE Confidence: 0.9090125

00:27:13.014 --> 00:27:15.318 And perhaps due to where the

NOTE Confidence: 0.9090125

00:27:15.318 --> 00:27:18.029 nature of the stress originates,

NOTE Confidence: 0.9090125

00:27:18.030 --> 00:27:19.870 small molecule stress within the

NOTE Confidence: 0.9090125

00:27:19.870 --> 00:27:21.710 mitochondria creates one flavor of

NOTE Confidence: 0.9090125

00:27:21.765 --> 00:27:24.615 the UPR and then a more physiological

NOTE Confidence: 0.9090125

00:27:24.615 --> 00:27:26.346 potentially you know potentially

NOTE Confidence: 0.9090125

00:27:26.346 --> 00:27:28.406 physiological regulator of MiTo UPR

NOTE Confidence: 0.9090125

00:27:28.406 --> 00:27:30.698 coming from the outside into the

NOTE Confidence: 0.9090125

00:27:30.698 --> 00:27:32.804 mitochondria changes the way that the

NOTE Confidence: 0.9090125

00:27:32.872 --> 00:27:35.060 cells respond to this pathway Okay.

NOTE Confidence: 0.9090125

00:27:35.060 --> 00:27:37.640 So what happens if we eliminate

NOTE Confidence: 0.9090125

00:27:37.640 --> 00:27:41.110 ATF5 in throughout the system?

NOTE Confidence: 0.9090125

00:27:41.110 --> 00:27:42.870 I won't go through all of the data.

NOTE Confidence: 0.9090125

00:27:42.870 --> 00:27:45.270 We've also done this in the a TF5

NOTE Confidence: 0.9090125

00:27:45.270 --> 00:27:47.160 deficient mouse and if you have no

NOTE Confidence: 0.9090125

00:27:47.160 --> 00:27:49.429 oncogene in the a TF5 deficient mouse,

NOTE Confidence: 0.9090125

00:27:49.430 --> 00:27:51.310 the skin is perfectly fine.

NOTE Confidence: 0.9090125

00:27:51.310 --> 00:27:53.610 There might be a slight

NOTE Confidence: 0.9090125

00:27:53.610 --> 00:27:55.250 inflammatory component in the skin,

NOTE Confidence: 0.9090125

00:27:55.250 --> 00:27:56.430 but it's pretty minimal.

NOTE Confidence: 0.9090125

00:27:56.430 --> 00:27:58.308 You get normal Melania type function.

NOTE Confidence: 0.9090125

00:27:58.310 --> 00:28:00.614 No issues at all here.

NOTE Confidence: 0.9090125

00:28:00.614 --> 00:28:02.034 If we take melanocytes and

NOTE Confidence: 0.9090125

00:28:02.034 --> 00:28:03.450 then we introduce B RAF,

NOTE Confidence: 0.9090125

00:28:03.450 --> 00:28:05.570 we silence ATF5 or we do the combination,

NOTE Confidence: 0.9090125

00:28:05.570 --> 00:28:07.770 you get some interesting responses.

NOTE Confidence: 0.9090125

00:28:07.770 --> 00:28:11.268 Of course we can silence ATF5ATF4 and chop.

NOTE Confidence: 0.9090125

00:28:11.268 --> 00:28:12.898 Remember I mentioned earlier that

NOTE Confidence: 0.9090125

00:28:12.898 --> 00:28:14.850 it's usual that they work together?

NOTE Confidence: 0.9090125

00:28:14.850 --> 00:28:16.490 There's always a marked compensation

NOTE Confidence: 0.9090125

00:28:16.490 --> 00:28:18.929 when one or the other is eliminated

NOTE Confidence: 0.9090125

00:28:18.930 --> 00:28:20.890 and you can see that a TF5IS induced

NOTE Confidence: 0.9090125

00:28:20.890 --> 00:28:23.821 a bit when we silence ATF5 or ATF4

NOTE Confidence: 0.9090125

00:28:23.821 --> 00:28:26.083 is induced when you silence ATF5.

NOTE Confidence: 0.9090125

00:28:26.090 --> 00:28:28.704 Chop doesn't move too much and importantly

NOTE Confidence: 0.9090125

00:28:28.704 --> 00:28:30.614 B REF signaling doesn't change.

NOTE Confidence: 0.9090125

00:28:30.620 --> 00:28:32.965 We needed to show that when you

NOTE Confidence: 0.9090125

00:28:32.965 --> 00:28:34.377 silence the mitochondrial unfolded

NOTE Confidence: 0.9090125

00:28:34.377 --> 00:28:36.107 protein response pathway that you

NOTE Confidence: 0.9090125

00:28:36.107 --> 00:28:38.585 don't now lose a BRB ref or six energy

NOTE Confidence: 0.9090125

00:28:38.585 --> 00:28:40.312 signaling and we don't lose it at the

NOTE Confidence: 0.9090125

00:28:40.312 --> 00:28:41.705 level of the expression and we also

NOTE Confidence: 0.9090125

00:28:41.754 --> 00:28:43.259 don't lose the downstream targets.

NOTE Confidence: 0.93345517

00:28:45.300 --> 00:28:47.490 What's curious is you do lose

NOTE Confidence: 0.93345517

00:28:47.490 --> 00:28:48.220 mitochondrial expansion,

NOTE Confidence: 0.93345517

00:28:48.220 --> 00:28:49.684 suggesting that when you put B

NOTE Confidence: 0.93345517

00:28:49.684 --> 00:28:52.077 ref into a cell and you get all

NOTE Confidence: 0.93345517

00:28:52.077 --> 00:28:53.100 those beautiful mitochondria.

NOTE Confidence: 0.93345517

00:28:53.100 --> 00:28:56.592 If you don't have a TF5 that doesn't happen.

NOTE Confidence: 0.93345517

00:28:56.600 --> 00:28:58.637 The reason why is you lose PGC

NOTE Confidence: 0.93345517

00:28:58.637 --> 00:29:01.022 1A and these mitochondria also

NOTE Confidence: 0.93345517

00:29:01.022 --> 00:29:03.731 don't engage an increase in their

NOTE Confidence: 0.93345517

00:29:03.731 --> 00:29:05.571 delta PSI and TMRE staining is

NOTE Confidence: 0.93345517

00:29:05.571 --> 00:29:07.113 a nice surrogate to look at.

NOTE Confidence: 0.93345517

00:29:07.120 --> 00:29:09.560 Oxygen consumption or proton pumping

NOTE Confidence: 0.93345517

00:29:09.560 --> 00:29:12.320 within this network and B REF

NOTE Confidence: 0.93345517

00:29:12.320 --> 00:29:14.560 V600E will increase TMRE staining.

NOTE Confidence: 0.93345517

00:29:14.560 --> 00:29:16.120 And if you do this in the presence

NOTE Confidence: 0.93345517

00:29:16.120 --> 00:29:17.773 of a TF5 you basically just

NOTE Confidence: 0.93345517

00:29:17.773 --> 00:29:19.278 flatline and there's no change.

NOTE Confidence: 0.9333408

00:29:21.560 --> 00:29:23.096 Same thing happens with some of

NOTE Confidence: 0.9333408

00:29:23.096 --> 00:29:24.709 these data sets that I showed

NOTE Confidence: 0.9333408

00:29:24.709 --> 00:29:26.950 you earlier before where you have

NOTE Confidence: 0.9333408

00:29:26.950 --> 00:29:29.350 this marked shift in glycolysis.

NOTE Confidence: 0.9333408

00:29:29.350 --> 00:29:31.630 Loss in glycolysis and a marked

NOTE Confidence: 0.9333408

00:29:31.630 --> 00:29:33.150 increase in mitochondrial function

NOTE Confidence: 0.9333408

00:29:33.150 --> 00:29:36.061 doesn't happen in the absence of a TFIB

NOTE Confidence: 0.9333408

00:29:36.061 --> 00:29:38.616 REF cannot remodel the mitochondrial

NOTE Confidence: 0.9333408

00:29:38.616 --> 00:29:41.200 network to erode glycolysis or

NOTE Confidence: 0.9333408

00:29:41.200 --> 00:29:43.300 increase mitochondrial function if

NOTE Confidence: 0.9333408

00:29:43.300 --> 00:29:46.548 the MiTo UPR pathway is not active.

NOTE Confidence: 0.9333408

00:29:46.550 --> 00:29:49.998 And also looking at the state 3 driven

NOTE Confidence: 0.9333408

00:29:49.998 --> 00:29:51.954 respirations, complex one changes,

NOTE Confidence: 0.9333408

00:29:51.954 --> 00:29:53.478 complex 2 changes,

NOTE Confidence: 0.9333408

00:29:53.480 --> 00:29:55.676 all of those are also minimized

NOTE Confidence: 0.9333408

00:29:55.676 --> 00:29:57.819 in this presence of no ATFI,

NOTE Confidence: 0.9333408

00:29:57.819 --> 00:30:00.114 suggesting that in order for

NOTE Confidence: 0.9333408

00:30:00.114 --> 00:30:03.240 B ref to expand mitochondria,

NOTE Confidence: 0.9333408

00:30:03.240 --> 00:30:04.186 remodel them,

NOTE Confidence: 0.9333408

00:30:04.186 --> 00:30:06.551 increase complex one and complex

NOTE Confidence: 0.9333408

00:30:06.551 --> 00:30:08.880 2 activity erode glycolysis,

NOTE Confidence: 0.9333408

00:30:08.880 --> 00:30:10.780 it must activate this organelle

NOTE Confidence: 0.9333408

00:30:10.780 --> 00:30:11.920 quality control program.

NOTE Confidence: 0.93046325

00:30:14.280 --> 00:30:17.702 And if we look at some of the

NOTE Confidence: 0.93046325

00:30:17.702 --> 00:30:20.252 reasons as to why we see this marked

NOTE Confidence: 0.93046325

00:30:20.252 --> 00:30:22.500 increase in mitochondrial function,

NOTE Confidence: 0.93046325

00:30:22.500 --> 00:30:24.948 what you'll notice is that B RAF will

NOTE Confidence: 0.93046325

00:30:24.948 --> 00:30:27.860 induce expression of or replication of

NOTE Confidence: 0.93046325

00:30:27.860 --> 00:30:30.060 the mitochondrial genome most of the time.

NOTE Confidence: 0.93046325

00:30:30.060 --> 00:30:31.860 You know, people who study the nucleus,

NOTE Confidence: 0.93046325

00:30:31.860 --> 00:30:33.974 there's like 2 copies of most genes.

NOTE Confidence: 0.93046325

00:30:33.980 --> 00:30:35.816 People who study the mitochondrial genome,

NOTE Confidence: 0.93046325

00:30:35.820 --> 00:30:37.570 you can have anywhere between 0 copies

NOTE Confidence: 0.93046325

00:30:37.570 --> 00:30:39.910 of the genes because your red blood cells

NOTE Confidence: 0.93046325

00:30:39.910 --> 00:30:41.900 don't have mitochondria or certain muscles,

NOTE Confidence: 0.93046325

00:30:41.900 --> 00:30:45.134 and neurons have thousands of copies of

NOTE Confidence: 0.93046325

00:30:45.134 --> 00:30:46.816 mitochondrial genome and then melanocytes.

NOTE Confidence: 0.93046325

00:30:46.816 --> 00:30:48.580 They kind of are in the middle.

NOTE Confidence: 0.93046325

00:30:48.580 --> 00:30:51.076 And you see the V ref can induce

NOTE Confidence: 0.93046325

00:30:51.076 --> 00:30:53.226 the levels of mitochondrial DNA.

NOTE Confidence: 0.93046325

00:30:53.226 --> 00:30:54.942 And this doesn't happen in the

NOTE Confidence: 0.93046325

00:30:54.942 --> 00:30:56.982 absence of a TF5, the T fan,

NOTE Confidence: 0.93046325

00:30:56.982 --> 00:30:59.040 the PGC 1A results are held consistent

NOTE Confidence: 0.93046325

00:30:59.100 --> 00:31:01.260 and if you also look at the expression

NOTE Confidence: 0.93046325

00:31:01.260 --> 00:31:03.840 of all of these different genes that

NOTE Confidence: 0.93046325

00:31:03.840 --> 00:31:05.820 arise from the mitochondrial genome,

NOTE Confidence: 0.93046325

00:31:05.820 --> 00:31:09.540 they're all lost when a TF5 isn't there.

NOTE Confidence: 0.93046325

00:31:09.540 --> 00:31:12.179 So we picked these genes to represent.

NOTE Confidence: 0.93046325

00:31:12.180 --> 00:31:13.368 It's not comprehensive.

NOTE Confidence: 0.93046325

00:31:13.368 --> 00:31:15.744 You know there are 13 proteins,

NOTE Confidence: 0.93046325

00:31:15.750 --> 00:31:17.406 there are rivals on the RN A's and

NOTE Confidence: 0.93046325

00:31:17.406 --> 00:31:19.440 all of the TRN A's are encoded by

NOTE Confidence: 0.93046325

00:31:19.440 --> 00:31:20.850 the mitochondrial genome and this

NOTE Confidence: 0.93046325

00:31:20.900 --> 00:31:22.285 is just representations of those

NOTE Confidence: 0.93046325

00:31:22.285 --> 00:31:23.670 so that it looks nice.

NOTE Confidence: 0.9287157

00:31:26.310 --> 00:31:29.950 And then finally, what we can see

NOTE Confidence: 0.9287157

00:31:29.950 --> 00:31:33.240 is all of these negative outcomes

NOTE Confidence: 0.9287157

00:31:33.240 --> 00:31:36.420 that occur because of B ref induced

NOTE Confidence: 0.9287157

00:31:36.420 --> 00:31:38.076 mitochondrial remodeling also don't

NOTE Confidence: 0.9287157

00:31:38.076 --> 00:31:40.960 occur if we don't have this orbinal

NOTE Confidence: 0.9287157

00:31:40.960 --> 00:31:42.564 quality control program activated.

NOTE Confidence: 0.9287157

00:31:42.570 --> 00:31:43.914 You can see that the MiTo

NOTE Confidence: 0.9287157

00:31:43.914 --> 00:31:44.810 Sox intensity goes away.

NOTE Confidence: 0.9287157

00:31:44.810 --> 00:31:46.874 This is looking at the comparing

NOTE Confidence: 0.9287157

00:31:46.874 --> 00:31:49.170 the Gray and the green lines.

NOTE Confidence: 0.9287157

00:31:49.170 --> 00:31:51.536 The same thing is there for the

NOTE Confidence: 0.9287157

00:31:51.536 --> 00:31:53.607 protein oxidation and the same thing

NOTE Confidence: 0.9287157

00:31:53.607 --> 00:31:55.924 is there for for the lipid oxidation.

NOTE Confidence: 0.9287157

00:31:55.930 --> 00:31:59.200 And if you look at the activation of

NOTE Confidence: 0.9287157

00:31:59.200 --> 00:32:01.965 the MiTo UPR pathway in this situation,

NOTE Confidence: 0.9287157

00:32:01.970 --> 00:32:05.085 you can also see that the combination

NOTE Confidence: 0.9287157

00:32:05.090 --> 00:32:06.850 completely ablates the activation

NOTE Confidence: 0.9287157

00:32:06.850 --> 00:32:08.170 of this pathway.

NOTE Confidence: 0.9287157

00:32:08.170 --> 00:32:10.070 So it's a really interesting

NOTE Confidence: 0.9287157

00:32:10.070 --> 00:32:12.136 phenomenon that you think your FP6

NOTE Confidence: 0.9287157

00:32:12.136 --> 00:32:13.228 under these signaling remodels,

NOTE Confidence: 0.9287157

00:32:13.230 --> 00:32:13.830 mitochondrial function,

NOTE Confidence: 0.9287157

00:32:13.830 --> 00:32:15.630 you get all of these mitochondrial,

NOTE Confidence: 0.9287157

00:32:15.630 --> 00:32:18.630 all these activities are engaged.

NOTE Confidence: 0.9287157

00:32:18.630 --> 00:32:19.850 The mitochondrial network tries

NOTE Confidence: 0.9287157

00:32:19.850 --> 00:32:21.070 to keep itself happy,

NOTE Confidence: 0.9287157

00:32:21.070 --> 00:32:22.980 tries to repair and restore

NOTE Confidence: 0.9287157

00:32:22.980 --> 00:32:24.508 and replenish the network,

NOTE Confidence: 0.9287157

00:32:24.510 --> 00:32:26.478 but it actually has a deleterious

NOTE Confidence: 0.9287157

00:32:26.478 --> 00:32:27.790 effect to the cell,

NOTE Confidence: 0.9287157

00:32:27.790 --> 00:32:30.540 suggesting that all quality control

NOTE Confidence: 0.9287157

00:32:30.540 --> 00:32:33.290 programs are not always necessarily

NOTE Confidence: 0.9287157

00:32:33.375 --> 00:32:35.469 to the benefit of the cell.

NOTE Confidence: 0.9287157

00:32:35.470 --> 00:32:39.766 So let's move away a little bit

NOTE Confidence: 0.9287157

00:32:39.766 --> 00:32:41.270 from human epidermal melanocytes.

NOTE Confidence: 0.9287157

00:32:41.270 --> 00:32:42.677 All of that work I just showed

NOTE Confidence: 0.9287157

00:32:42.677 --> 00:32:44.309 you was in primary melanocytes.

NOTE Confidence: 0.9287157

00:32:44.310 --> 00:32:46.510 And, you know, they're expensive,

NOTE Confidence: 0.9287157

00:32:46.510 --> 00:32:47.950 they're difficult to culture,

NOTE Confidence: 0.9287157

00:32:47.950 --> 00:32:48.886 they're slow growing.

NOTE Confidence: 0.9287157

00:32:48.886 --> 00:32:50.902 If you ever have to do mitochondrial

NOTE Confidence: 0.9287157

00:32:50.902 --> 00:32:52.468 metabolism research with melanocytes,

NOTE Confidence: 0.9287157

00:32:52.470 --> 00:32:53.784 you have to wait two months

NOTE Confidence: 0.9287157

00:32:53.784 --> 00:32:55.390 to get all the dishes grown,

NOTE Confidence: 0.9287157

00:32:55.390 --> 00:32:56.350 and then they're always finessed.

NOTE Confidence: 0.9287157

00:32:56.350 --> 00:32:57.589 In the absence of the Aqua gene,

NOTE Confidence: 0.9287157

00:32:57.590 --> 00:32:59.190 You know,

NOTE Confidence: 0.9287157

00:32:59.190 --> 00:33:00.870 we can move to some different models.

NOTE Confidence: 0.9287157

00:33:00.870 --> 00:33:04.321 And these are a series of Melanoma

NOTE Confidence: 0.9287157

00:33:04.321 --> 00:33:07.311 sun lines that are derived

NOTE Confidence: 0.9287157

00:33:07.311 --> 00:33:10.127 from primary Melanoma lesions.

NOTE Confidence: 0.9287157

00:33:10.130 --> 00:33:12.498 They're all be wrapped in 600D positive.

NOTE Confidence: 0.9287157

00:33:12.498 --> 00:33:14.682 And what you'll notice is all

NOTE Confidence: 0.9287157

00:33:14.682 --> 00:33:16.395 of them have statistically

NOTE Confidence: 0.9287157

00:33:16.395 --> 00:33:19.125 significant high levels of a TF5,

NOTE Confidence: 0.9287157

00:33:19.130 --> 00:33:21.314 both at the level of mRNA

NOTE Confidence: 0.9287157

00:33:21.314 --> 00:33:22.690 and also with protein.

NOTE Confidence: 0.9287157

00:33:22.690 --> 00:33:24.570 And if you look at the other markers,

NOTE Confidence: 0.9287157

00:33:24.570 --> 00:33:25.562 a TF4 and chop,

NOTE Confidence: 0.9287157

00:33:25.562 --> 00:33:27.450 they're kind of all over the place.

NOTE Confidence: 0.9287157

00:33:27.450 --> 00:33:28.806 Some of them have high levels,

NOTE Confidence: 0.9287157

00:33:28.810 --> 00:33:30.568 some of them have no change.

NOTE Confidence: 0.9287157

00:33:30.570 --> 00:33:33.062 And so this started to suggest

NOTE Confidence: 0.9287157

00:33:33.062 --> 00:33:36.506 to us that once you get through

NOTE Confidence: 0.9287157

00:33:36.510 --> 00:33:38.410 the escape from opportunities,

NOTE Confidence: 0.9287157

00:33:38.410 --> 00:33:42.304 in essence you have this addiction to the

NOTE Confidence: 0.9287157

00:33:42.304 --> 00:33:44.709 mitochondrial UPR to maintain survival.

NOTE Confidence: 0.9287157

00:33:44.710 --> 00:33:48.614 So what we did was we looked at all

NOTE Confidence: 0.9287157

00:33:48.614 --> 00:33:50.547 these cell lines we could silence

NOTE Confidence: 0.9287157

00:33:50.550 --> 00:33:52.428 ATF5ATF4 and chopping these cell lines.

NOTE Confidence: 0.9287157

00:33:52.430 --> 00:33:55.802 And what you'll notice is in contrast

NOTE Confidence: 0.9287157

00:33:55.802 --> 00:33:58.238 to the Melania site populations that

NOTE Confidence: 0.9287157

00:33:58.238 --> 00:34:00.942 are primary here this the levels

NOTE Confidence: 0.9287157

00:34:00.942 --> 00:34:02.758 of these cooperating transcription

NOTE Confidence: 0.9287157

00:34:02.758 --> 00:34:04.889 factors are quite consistent.

NOTE Confidence: 0.9287157

00:34:04.890 --> 00:34:06.969 So in almost all of the literature,

NOTE Confidence: 0.9287157

00:34:06.970 --> 00:34:10.530 when you silence ATF5ATF4 and chop skyrocket,

NOTE Confidence: 0.9287157

00:34:10.530 --> 00:34:13.743 if you silence chop a TF5 and

NOTE Confidence: 0.9287157

00:34:13.743 --> 00:34:15.210 ATF4 skyrocket here,

NOTE Confidence: 0.9287157

00:34:15.210 --> 00:34:17.538 you silence one the other stay

NOTE Confidence: 0.9287157

00:34:17.538 --> 00:34:18.554 pretty pretty consistent.

NOTE Confidence: 0.9287157

00:34:18.554 --> 00:34:21.050 And that's the same for all of these.

NOTE Confidence: 0.9287157

00:34:21.050 --> 00:34:23.360 And this slowly led us to believe

NOTE Confidence: 0.9287157

00:34:23.360 --> 00:34:25.492 that there was a unique dependency

NOTE Confidence: 0.9287157

00:34:25.492 --> 00:34:26.964 on the ATF5 pathway,

NOTE Confidence: 0.9287157

00:34:26.964 --> 00:34:28.849 and that these transcription factors,

NOTE Confidence: 0.9287157

00:34:28.850 --> 00:34:31.380 while throughout the classical literature,

NOTE Confidence: 0.9287157

00:34:31.380 --> 00:34:32.222 usually cooperate,

NOTE Confidence: 0.9287157

00:34:32.222 --> 00:34:34.327 there can be instances where

NOTE Confidence: 0.9287157

00:34:34.327 --> 00:34:35.860 they don't have to.

NOTE Confidence: 0.9287157

00:34:35.860 --> 00:34:36.420 And

NOTE Confidence: 0.9262983

00:34:38.940 --> 00:34:40.160 I promise, this is the

NOTE Confidence: 0.9262983

00:34:40.160 --> 00:34:41.380 worst slide I've ever made.

NOTE Confidence: 0.9262983

00:34:41.380 --> 00:34:42.298 I hope you agree with me.

NOTE Confidence: 0.9262983

00:34:42.300 --> 00:34:44.736 All right, it's busy, it's dense,

NOTE Confidence: 0.9262983

00:34:44.740 --> 00:34:45.780 and every time I present,

NOTE Confidence: 0.9262983

00:34:45.780 --> 00:34:47.292 I think to myself, I need to rebuild it.

NOTE Confidence: 0.9262983

00:34:47.300 --> 00:34:49.379 And then I just can't be bothered.

NOTE Confidence: 0.9262983

00:34:49.380 --> 00:34:51.335 I don't know why. So anyway,

NOTE Confidence: 0.9262983

00:34:51.335 --> 00:34:54.175 this is just one of those cell lines.

NOTE Confidence: 0.9262983

00:34:54.180 --> 00:34:55.422 I had a master's student who

NOTE Confidence: 0.9262983

00:34:55.422 --> 00:34:56.739 worked with me for two years.

NOTE Confidence: 0.9262983

00:34:56.740 --> 00:34:58.882 He had the seven cell lines that

NOTE Confidence: 0.9262983

00:34:58.882 --> 00:35:00.547 are derived from primary Melanoma

NOTE Confidence: 0.9262983

00:35:00.547 --> 00:35:02.913 lesions and I made him go through

NOTE Confidence: 0.9262983

00:35:02.913 --> 00:35:05.126 all of these assays 7 times over.

NOTE Confidence: 0.9262983

00:35:05.130 --> 00:35:05.970 OK and all the cell lines.

NOTE Confidence: 0.9262983

00:35:05.970 --> 00:35:07.296 And he categorized all of them

NOTE Confidence: 0.9262983

00:35:07.296 --> 00:35:08.450 and cataloged all of them.

NOTE Confidence: 0.9262983

00:35:08.450 --> 00:35:09.806 This is just one slide that

NOTE Confidence: 0.9262983

00:35:09.806 --> 00:35:11.330 shows one of the cell lines.

NOTE Confidence: 0.9262983

00:35:11.330 --> 00:35:12.730 This is the UP model.

NOTE Confidence: 0.9262983

00:35:12.730 --> 00:35:15.488 It's another V Rep P600E driven system.

NOTE Confidence: 0.9262983

00:35:15.490 --> 00:35:19.130 Of course they have basal ATF5 levels.

NOTE Confidence: 0.9262983

00:35:19.130 --> 00:35:22.325 They don't have that much chop or a TF4.

NOTE Confidence: 0.9262983

00:35:22.330 --> 00:35:24.090 And if you silence ATF5,

NOTE Confidence: 0.9262983

00:35:24.090 --> 00:35:26.426 chop and a TF4 don't change very much.

NOTE Confidence: 0.9262983

00:35:26.430 --> 00:35:28.295 What's interesting is if you

NOTE Confidence: 0.9262983

00:35:28.295 --> 00:35:30.790 silence ATF5 in these cells,

NOTE Confidence: 0.9262983

00:35:30.790 --> 00:35:33.126 P21P16 levels increase very

NOTE Confidence: 0.9262983

00:35:33.126 --> 00:35:35.420 quickly and you get this really

NOTE Confidence: 0.9262983

00:35:35.420 --> 00:35:37.639 beautiful expansion of the cell and

NOTE Confidence: 0.9262983

00:35:37.639 --> 00:35:39.427 of cellular senescent phenotype.

NOTE Confidence: 0.9262983

00:35:39.430 --> 00:35:40.585 You're going to see the

NOTE Confidence: 0.9262983

00:35:40.585 --> 00:35:41.509 mitochondria look different here,

NOTE Confidence: 0.9262983

00:35:41.510 --> 00:35:42.398 there's a different architecture

NOTE Confidence: 0.9262983

00:35:42.398 --> 00:35:44.070 to these than I showed you earlier.

NOTE Confidence: 0.9262983

00:35:44.070 --> 00:35:46.527 In the primary melanosite you get those

NOTE Confidence: 0.9262983

00:35:46.527 --> 00:35:47.986 beautiful long connected mitochondria

NOTE Confidence: 0.9262983

00:35:47.986 --> 00:35:50.092 and here it's slightly different and

NOTE Confidence: 0.9262983

00:35:50.092 --> 00:35:52.357 we're going to talk about in the

NOTE Confidence: 0.9262983

00:35:52.357 --> 00:35:55.438 second-half of this why that's the case.

NOTE Confidence: 0.9262983

00:35:55.440 --> 00:35:56.928 You can also see that they

NOTE Confidence: 0.9262983

00:35:56.928 --> 00:35:57.920 have an opposite effect.

NOTE Confidence: 0.9262983

00:35:57.920 --> 00:35:59.296 You sign on to a TF5 and now

NOTE Confidence: 0.9262983

00:35:59.296 --> 00:36:00.839 you get mitochondrial expansion,

NOTE Confidence: 0.9262983

00:36:00.840 --> 00:36:01.824 you get T FM,

NOTE Confidence: 0.9262983

00:36:01.824 --> 00:36:03.220 you get PGC 1A,

NOTE Confidence: 0.9262983

00:36:03.220 --> 00:36:05.020 you get mitochondrial enhancement

NOTE Confidence: 0.9262983

00:36:05.020 --> 00:36:05.920 of function.

NOTE Confidence: 0.9262983

00:36:05.920 --> 00:36:08.920 And you also see this increase in Okar.

NOTE Confidence: 0.9262983

00:36:08.920 --> 00:36:11.062 So these cells are consuming more oxygen

NOTE Confidence: 0.9262983

00:36:11.062 --> 00:36:12.400 through the mitochondrial network.

NOTE Confidence: 0.9287162

00:36:14.800 --> 00:36:16.036 It's kind of a fun control.

NOTE Confidence: 0.9287162

00:36:16.040 --> 00:36:17.832 What we did was we also stimulate

NOTE Confidence: 0.9287162

00:36:17.832 --> 00:36:19.530 these cells with GTPP which is

NOTE Confidence: 0.9287162

00:36:19.530 --> 00:36:21.080 again is that small molecule.

NOTE Confidence: 0.9287162

00:36:21.080 --> 00:36:23.048 Take a cancer cell that we think is

NOTE Confidence: 0.9287162

00:36:23.048 --> 00:36:25.121 addicted to a TF5 signaling and now

NOTE Confidence: 0.9287162

00:36:25.121 --> 00:36:27.120 hyperactivate the pathway and what happens?

NOTE Confidence: 0.9287162

00:36:27.120 --> 00:36:29.495 The exact opposite is mitochondria

NOTE Confidence: 0.9287162

00:36:29.495 --> 00:36:32.300 collapse and function so it gives you

NOTE Confidence: 0.9287162

00:36:32.300 --> 00:36:34.959 this concept of minor hormesis where this

NOTE Confidence: 0.9287162

00:36:34.959 --> 00:36:37.559 tonic mitochondrial function is necessary.

NOTE Confidence: 0.9287162

00:36:37.560 --> 00:36:39.616 Tonic mitochondrial stress signaling

NOTE Confidence: 0.9287162

00:36:39.616 --> 00:36:42.186 is necessary to maintain survival

NOTE Confidence: 0.9287162

00:36:42.186 --> 00:36:44.849 and if you hyperactivate the pathway

NOTE Confidence: 0.9287162

00:36:44.849 --> 00:36:46.513 now the cell crashes.

NOTE Confidence: 0.9287162

00:36:46.520 --> 00:36:49.536 So we've done this in seven cell lines

NOTE Confidence: 0.9287162

00:36:49.540 --> 00:36:51.020 and we've also started to move into some

NOTE Confidence: 0.9287162

00:36:51.020 --> 00:36:52.620 of the metastatic cell lines as well.

NOTE Confidence: 0.9287162

00:36:52.620 --> 00:36:54.700 Everyone knows STML series,

NOTE Confidence: 0.9287162

00:36:54.700 --> 00:36:56.884 A370, Fives, all of these.

NOTE Confidence: 0.9287162

00:36:56.884 --> 00:36:59.665 Once you enter the metastatic lesion and

NOTE Confidence: 0.9287162

00:36:59.665 --> 00:37:01.660 you're no longer at the primary site.

NOTE Confidence: 0.9287162

00:37:01.660 --> 00:37:03.340 This requirement really goes by,

NOTE Confidence: 0.92890567

00:37:07.620 --> 00:37:10.388 this is starting to look at some of

NOTE Confidence: 0.92890567

00:37:10.388 --> 00:37:14.240 the ATF5 expression levels in patient

NOTE Confidence: 0.92890567

00:37:14.240 --> 00:37:16.876 samples comparing here normal skin,

NOTE Confidence: 0.92890567

00:37:16.876 --> 00:37:19.296 dysplastic Nevis and intradermal nevi.

NOTE Confidence: 0.92890567

00:37:19.300 --> 00:37:21.736 We're still in the process of going

NOTE Confidence: 0.92890567

00:37:21.736 --> 00:37:24.545 through all of the T123 and four

NOTE Confidence: 0.92890567

00:37:24.545 --> 00:37:27.020 samples from the Melanoma patients.

NOTE Confidence: 0.92890567

00:37:27.020 --> 00:37:30.060 They are also statistically increased,

NOTE Confidence: 0.92890567

00:37:30.060 --> 00:37:31.383 but I'm not showing them here because

NOTE Confidence: 0.92890567

00:37:31.383 --> 00:37:32.698 we don't have the study done yet.

NOTE Confidence: 0.92890567

00:37:32.700 --> 00:37:34.268 But what you can see is as you

NOTE Confidence: 0.92890567

00:37:34.268 --> 00:37:35.820 start to enter the progression,

NOTE Confidence: 0.92890567

00:37:35.820 --> 00:37:38.034 you start to see this enhancement

NOTE Confidence: 0.92890567

00:37:38.034 --> 00:37:40.757 of the levels of a TF5 that

NOTE Confidence: 0.92890567

00:37:40.757 --> 00:37:42.697 are detected within the lesion.

NOTE Confidence: 0.92890567

00:37:42.700 --> 00:37:46.300 And what we did then was we cooperate.

NOTE Confidence: 0.92890567

00:37:46.300 --> 00:37:48.300 We collaborated with A

NOTE Confidence: 0.9276607

00:37:50.440 --> 00:37:53.564 and I'm a dermatologist at NYU,

NOTE Confidence: 0.9276607

00:37:53.564 --> 00:37:56.984 Julie Chile Solavy and we

NOTE Confidence: 0.9276607

00:37:56.984 --> 00:37:59.720 sequenced 51 Melanoma patients,

NOTE Confidence: 0.9276607

00:37:59.720 --> 00:38:02.037 all of which these are primary lesions.

NOTE Confidence: 0.9276607

00:38:02.040 --> 00:38:04.714 And then using the MiTo UPR signatures,

NOTE Confidence: 0.9276607

00:38:04.720 --> 00:38:07.478 we look to see how they clustered.

NOTE Confidence: 0.9276607

00:38:07.480 --> 00:38:08.439 And what you can see is this,

NOTE Confidence: 0.9276607

00:38:08.440 --> 00:38:09.520 this nice clustering.

NOTE Confidence: 0.9276607

00:38:09.520 --> 00:38:11.320 Looking at chopped a TF4,

NOTE Confidence: 0.9276607

00:38:11.320 --> 00:38:13.608 all of the targets are in blue and

NOTE Confidence: 0.9276607

00:38:13.608 --> 00:38:15.106 green respectively and then all of

NOTE Confidence: 0.9276607

00:38:15.106 --> 00:38:16.839 the targets of a TF5 are in red.

NOTE Confidence: 0.9276607

00:38:16.840 --> 00:38:19.360 We don't necessarily have to look at a

NOTE Confidence: 0.9276607

00:38:19.360 --> 00:38:21.280 TF5M RNA changes because remember this

NOTE Confidence: 0.9276607

00:38:21.280 --> 00:38:22.560 protein is constitutively expressed

NOTE Confidence: 0.9276607

00:38:22.612 --> 00:38:23.997 and goes between the organelles,

NOTE Confidence: 0.9276607

00:38:24.000 --> 00:38:26.310 so it doesn't have to be increased

NOTE Confidence: 0.9276607

00:38:26.310 --> 00:38:27.880 in terms of levels.

NOTE Confidence: 0.9276607

00:38:27.880 --> 00:38:29.599 But what you can see really nicely is this,

NOTE Confidence: 0.9276607

00:38:29.600 --> 00:38:30.800 this clustering arrangement.

NOTE Confidence: 0.9276607

00:38:30.800 --> 00:38:33.600 And what's curious is all the patients

NOTE Confidence: 0.9276607

00:38:33.662 --> 00:38:35.950 who are in green are the ones who

NOTE Confidence: 0.9276607

00:38:35.950 --> 00:38:37.328 developed high risk metastatic

NOTE Confidence: 0.9276607

00:38:37.328 --> 00:38:39.862 disease as well suggesting that this

NOTE Confidence: 0.9276607

00:38:39.862 --> 00:38:42.706 tonic activation of the meta UPR

NOTE Confidence: 0.9276607

00:38:42.706 --> 00:38:44.208 pathway could have some predictive

NOTE Confidence: 0.9276607

00:38:44.208 --> 00:38:45.750 ability to look at patients who

NOTE Confidence: 0.9276607

00:38:45.797 --> 00:38:47.255 have the highest risk of disease.

NOTE Confidence: 0.9293274

00:38:52.660 --> 00:38:57.976 OK. So, so far I showed you the

NOTE Confidence: 0.9293274

00:38:57.976 --> 00:38:59.600 transition between primary melanosite

NOTE Confidence: 0.9293274

00:38:59.673 --> 00:39:01.220 and B rap driven melanosite.

NOTE Confidence: 0.9293274

00:39:01.220 --> 00:39:03.140 These are stead images.

NOTE Confidence: 0.9293274

00:39:03.140 --> 00:39:04.384 I probably should have

NOTE Confidence: 0.9293274

00:39:04.384 --> 00:39:05.939 described this 40 slides ago.

NOTE Confidence: 0.9293274

00:39:05.940 --> 00:39:08.100 Looking at these are fixed cells,

NOTE Confidence: 0.9293274

00:39:08.100 --> 00:39:09.102 same with HP60.

NOTE Confidence: 0.9293274

00:39:09.102 --> 00:39:11.440 And then we take the images deconvolute

NOTE Confidence: 0.9293274

00:39:11.510 --> 00:39:13.550 and then make these beautiful movies

NOTE Confidence: 0.9293274

00:39:13.550 --> 00:39:15.650 of what the network looks like.

NOTE Confidence: 0.9293274

00:39:15.650 --> 00:39:17.210 Normal Milano sites,

NOTE Confidence: 0.9293274

00:39:17.210 --> 00:39:18.770 nice connected network,

NOTE Confidence: 0.9293274

00:39:18.770 --> 00:39:21.170 OIS Milano sites, still connected,

NOTE Confidence: 0.9293274

00:39:21.170 --> 00:39:23.370 but a lot more mitochondria.

NOTE Confidence: 0.9293274

00:39:23.370 --> 00:39:25.050 And then when you transform these cells,

NOTE Confidence: 0.9293274

00:39:25.050 --> 00:39:26.098 what do you see?

NOTE Confidence: 0.9293274

00:39:26.098 --> 00:39:28.370 All the mitochondria are kind of unconnected,

NOTE Confidence: 0.9293274

00:39:28.370 --> 00:39:30.248 disconnected, undergoing fragmentation,

NOTE Confidence: 0.9293274

00:39:30.248 --> 00:39:34.004 and they don't look very helpful.

NOTE Confidence: 0.9293274

00:39:34.010 --> 00:39:37.769 And I was always interested in knowing,

NOTE Confidence: 0.9293274

00:39:37.770 --> 00:39:39.835 you know, when my lad first started,

NOTE Confidence: 0.9293274

00:39:39.840 --> 00:39:42.012 we studied this transition from primary

NOTE Confidence: 0.9293274

00:39:42.012 --> 00:39:44.438 to transform because it was the easiest.

NOTE Confidence: 0.9293274

00:39:44.440 --> 00:39:47.113 We could do this in self culture very easily.

NOTE Confidence: 0.9293274

00:39:47.120 --> 00:39:48.960 This part here from primary to OIS was

NOTE Confidence: 0.9293274

00:39:48.960 --> 00:39:50.656 much more complicated and that's what

NOTE Confidence: 0.9293274

00:39:50.656 --> 00:39:52.438 we're just figuring out right now.

NOTE Confidence: 0.9293274

00:39:52.440 --> 00:39:53.595 In order to share it with you,

NOTE Confidence: 0.9293274

00:39:53.600 --> 00:39:55.098 let me tell you a little bit

NOTE Confidence: 0.9293274

00:39:55.098 --> 00:39:56.040 about this next step.

NOTE Confidence: 0.9293274

00:39:56.040 --> 00:39:58.968 So I mentioned earlier that money

NOTE Confidence: 0.9293274

00:39:58.968 --> 00:40:01.373 country are dynamic organelles and

NOTE Confidence: 0.9293274

00:40:01.373 --> 00:40:03.638 they undergo rounds of fusion.

NOTE Confidence: 0.9293274

00:40:03.640 --> 00:40:05.520 They undergo rounds of fission.

NOTE Confidence: 0.9293274

00:40:05.520 --> 00:40:07.290 And the reason why they do

NOTE Confidence: 0.9293274

00:40:07.290 --> 00:40:09.120 this is to exchange material.

NOTE Confidence: 0.9293274

00:40:09.120 --> 00:40:11.680 And if you look at a mitochondrial network,

NOTE Confidence: 0.9293274

00:40:11.680 --> 00:40:13.696 the network within a single cell

NOTE Confidence: 0.9293274

00:40:13.696 --> 00:40:15.920 tends to be quite homogeneous.

NOTE Confidence: 0.9293274

00:40:15.920 --> 00:40:17.968 They're always exchanging proteins,

NOTE Confidence: 0.9293274

00:40:17.968 --> 00:40:19.232 lipids, DNA, metabolites,

NOTE Confidence: 0.9293274

00:40:19.232 --> 00:40:22.240 and that keeps the network equal and happy.

NOTE Confidence: 0.9293274

00:40:22.240 --> 00:40:23.520 So when you image them,

NOTE Confidence: 0.9293274

00:40:23.520 --> 00:40:26.080 they all look the same.

NOTE Confidence: 0.9293274

00:40:26.080 --> 00:40:28.640 And what's responsible for this,

NOTE Confidence: 0.9293274

00:40:28.640 --> 00:40:29.394 of course,

NOTE Confidence: 0.9293274

00:40:29.394 --> 00:40:31.279 is the mitochondrial dynamics machinery.

NOTE Confidence: 0.9293274

00:40:31.280 --> 00:40:32.596 We're not going to talk about fusion.

NOTE Confidence: 0.9293274

00:40:32.600 --> 00:40:34.735 We're just going to talk about division.

NOTE Confidence: 0.9293274

00:40:34.740 --> 00:40:36.948 And the main protein that's responsible

NOTE Confidence: 0.9293274

00:40:36.948 --> 00:40:38.420 for mitochondrial division is

NOTE Confidence: 0.9293274

00:40:38.473 --> 00:40:40.338 dynamically related to protein one.

NOTE Confidence: 0.9293274

00:40:40.340 --> 00:40:41.939 It's normally cytosolic,

NOTE Confidence: 0.9293274

00:40:41.939 --> 00:40:45.137 you phosphorylate it to activate it,

NOTE Confidence: 0.9293274

00:40:45.140 --> 00:40:46.464 it as a monitor,

NOTE Confidence: 0.9293274

00:40:46.464 --> 00:40:47.457 goes to mitochondria,

NOTE Confidence: 0.9293274

00:40:47.460 --> 00:40:48.080 accumulates,

NOTE Confidence: 0.9293274

00:40:48.080 --> 00:40:51.180 generates decision machinery and then

NOTE Confidence: 0.9293274

00:40:51.180 --> 00:40:53.660 divides the mitochondrial network.

NOTE Confidence: 0.9293274

00:40:53.660 --> 00:40:55.670 And I became very interested in

NOTE Confidence: 0.9293274

00:40:55.670 --> 00:40:58.077 knowing how the Met Klinase pathway

NOTE Confidence: 0.9293274

00:40:58.077 --> 00:41:00.073 could alter mitochondrial dynamics

NOTE Confidence: 0.9293274

00:41:00.073 --> 00:41:02.470 because of this phenotype here.

NOTE Confidence: 0.9293274

00:41:02.470 --> 00:41:04.870 And that is, if you look at wild type maps,

NOTE Confidence: 0.9293274

00:41:04.870 --> 00:41:07.000 you see beautiful connected mitochondria

NOTE Confidence: 0.9293274

00:41:07.000 --> 00:41:09.110 and then when you fragment them,

NOTE Confidence: 0.9293274

00:41:09.110 --> 00:41:11.561 mitochondrial division stays

NOTE Confidence: 0.9293274

00:41:11.561 --> 00:41:14.829 much longer in cells.

NOTE Confidence: 0.9293274

00:41:14.830 --> 00:41:17.548 These cells always display a chronically

NOTE Confidence: 0.9293274

00:41:17.548 --> 00:41:18.907 divided mitochondrial network,

NOTE Confidence: 0.9293274

00:41:18.910 --> 00:41:21.030 the majority of cancer cells,

NOTE Confidence: 0.9293274

00:41:21.030 --> 00:41:22.630 and then compare those to

NOTE Confidence: 0.9293274

00:41:22.630 --> 00:41:24.230 the actual tissue of origin,

NOTE Confidence: 0.9293274

00:41:24.230 --> 00:41:26.435 you will see chronically divided

NOTE Confidence: 0.9293274

00:41:26.435 --> 00:41:27.758 mitochondrial networks in

NOTE Confidence: 0.9293274

00:41:27.758 --> 00:41:29.270 the majority of cases.

NOTE Confidence: 0.9293274

00:41:29.270 --> 00:41:31.502 You can see that the consequence

NOTE Confidence: 0.9293274

00:41:31.502 --> 00:41:34.939 of this of course is less oak car,

NOTE Confidence: 0.9293274

00:41:34.939 --> 00:41:37.800 less oxygen consumption at the basal level,

NOTE Confidence: 0.9293274

00:41:37.800 --> 00:41:38.655 the maximum level,

NOTE Confidence: 0.9293274

00:41:38.660 --> 00:41:41.892 and also a decrease in the ability for

NOTE Confidence: 0.9293274

00:41:41.892 --> 00:41:43.978 these mitochondria to generate a TP.

NOTE Confidence: 0.9293274

00:41:43.980 --> 00:41:47.148 And what we discovered in this paper was

NOTE Confidence: 0.9293274

00:41:47.148 --> 00:41:49.784 that when you put grass into a cell,

NOTE Confidence: 0.9293274

00:41:49.784 --> 00:41:51.289 you get activation of course

NOTE Confidence: 0.9293274

00:41:51.289 --> 00:41:53.098 of the math kinase pathway.

NOTE Confidence: 0.9293274

00:41:53.100 --> 00:41:55.116 And we identified that there's a

NOTE Confidence: 0.9293274

00:41:55.116 --> 00:41:57.085 phosphorylation site on D RP1AT

NOTE Confidence: 0.9293274

00:41:57.085 --> 00:42:00.210 series 616 that's activated by

NOTE Confidence: 0.9293274

00:42:00.210 --> 00:42:01.850 on the genuine kinase signaling.

NOTE Confidence: 0.9293274

00:42:01.850 --> 00:42:03.782 And this is what was responsible

NOTE Confidence: 0.9293274

00:42:03.782 --> 00:42:05.570 for actively dividing that network.

NOTE Confidence: 0.9293274

00:42:05.570 --> 00:42:07.010 If you looked into literature,

NOTE Confidence: 0.93488103

00:42:07.010 --> 00:42:08.290 616 in the human,

NOTE Confidence: 0.93488103

00:42:08.290 --> 00:42:11.626 and I think it's 5:30 or 592 in the mouse,

NOTE Confidence: 0.93488103

00:42:11.626 --> 00:42:13.396 this is the activating phosphorylation

NOTE Confidence: 0.93488103

00:42:13.396 --> 00:42:15.168 for the decision machinery.

NOTE Confidence: 0.93488103

00:42:15.170 --> 00:42:17.432 And if you add these targeted

NOTE Confidence: 0.93488103

00:42:17.432 --> 00:42:19.515 therapies to cells, you can turn

NOTE Confidence: 0.93488103

00:42:19.515 --> 00:42:21.090 off the phosphorylation in meths.

NOTE Confidence: 0.93488103

00:42:21.090 --> 00:42:24.410 And you can also see how you have

NOTE Confidence: 0.93488103

00:42:24.410 --> 00:42:26.760 the phosphorylation of D RP1 in green

NOTE Confidence: 0.93488103

00:42:26.760 --> 00:42:28.988 here and then it completely goes away

NOTE Confidence: 0.93488103

00:42:28.988 --> 00:42:31.390 when you treat with GSK from 120212

NOTE Confidence: 0.93488103

00:42:31.390 --> 00:42:33.778 or and you can see the difference

NOTE Confidence: 0.93488103

00:42:33.778 --> 00:42:34.930 in the money comes from network.

NOTE Confidence: 0.93488103

00:42:34.930 --> 00:42:37.360 Those are fragmented to beautifully

NOTE Confidence: 0.93488103

00:42:37.360 --> 00:42:39.530 connected within just a few hours actually

NOTE Confidence: 0.93051195

00:42:41.690 --> 00:42:43.553 and I'm not going to take you through all

NOTE Confidence: 0.93051195

00:42:43.553 --> 00:42:45.647 of this because this portion is published.

NOTE Confidence: 0.93051195

00:42:45.650 --> 00:42:48.960 What you can see is you can screen all of

NOTE Confidence: 0.93051195

00:42:48.960 --> 00:42:51.090 these, all of these targeted therapies.

NOTE Confidence: 0.93051195

00:42:51.090 --> 00:42:53.214 Here we're just using a 375 cells.

NOTE Confidence: 0.93051195

00:42:53.214 --> 00:42:55.386 The B ref is 600 positive.

NOTE Confidence: 0.93051195

00:42:55.390 --> 00:42:57.070 You can also do SKML 28,

NOTE Confidence: 0.93051195

00:42:57.070 --> 00:42:58.470 so you can hit them with Plexicon.

NOTE Confidence: 0.93051195

00:42:58.470 --> 00:43:00.464 You can hit them with the, you know,

NOTE Confidence: 0.93051195

00:43:00.464 --> 00:43:03.062 very old PD mech inhibitor GSK.

NOTE Confidence: 0.93051195

00:43:03.062 --> 00:43:06.430 You can also use or lot NIM to hit or B2.

NOTE Confidence: 0.93051195

00:43:06.430 --> 00:43:09.774 You get really beautiful rapid fusion of the

NOTE Confidence: 0.93051195

00:43:09.774 --> 00:43:12.066 mitochondria networks in all of these cases.

NOTE Confidence: 0.93051195

00:43:12.070 --> 00:43:13.426 And what they basically do is,

NOTE Confidence: 0.93051195

00:43:13.430 --> 00:43:15.500 of course they reduce the European

NOTE Confidence: 0.93051195

00:43:15.500 --> 00:43:17.350 1 phosphorylation to turn it off,

NOTE Confidence: 0.93051195

00:43:17.350 --> 00:43:20.052 but they also you lose D RP1

NOTE Confidence: 0.93051195

00:43:20.052 --> 00:43:21.740 protein and you lose message.

NOTE Confidence: 0.93051195

00:43:21.740 --> 00:43:23.760 And what happens is, guess what,

NOTE Confidence: 0.93051195

00:43:23.760 --> 00:43:25.200 all those chronically divided

NOTE Confidence: 0.93051195

00:43:25.200 --> 00:43:26.280 mitochondria now fuse.

NOTE Confidence: 0.93051195

00:43:26.280 --> 00:43:27.712 And when they fuse,

NOTE Confidence: 0.93051195

00:43:27.712 --> 00:43:30.372 they exchange materials and now all the

NOTE Confidence: 0.93051195

00:43:30.372 --> 00:43:33.000 mitochondrial function goes back to normal.

NOTE Confidence: 0.93051195

00:43:33.000 --> 00:43:35.130 So it's a really dynamic process

NOTE Confidence: 0.93051195

00:43:35.130 --> 00:43:37.320 and it's really amazing to look at.

NOTE Confidence: 0.93051195

00:43:37.320 --> 00:43:39.686 And if you go into patient sections

NOTE Confidence: 0.93051195

00:43:39.690 --> 00:43:41.574 here we're just looking at melanomas

NOTE Confidence: 0.93051195

00:43:41.574 --> 00:43:43.954 and C2 using the V E1 antibody against

NOTE Confidence: 0.93051195

00:43:43.954 --> 00:43:45.966 V ref P600E and then screening

NOTE Confidence: 0.93051195

00:43:45.966 --> 00:43:47.811 using a commercially available mouse

NOTE Confidence: 0.93051195

00:43:47.811 --> 00:43:50.326 antibody that also picks up the human

NOTE Confidence: 0.93051195

00:43:50.326 --> 00:43:51.370 phosphorylation adjacent sections

NOTE Confidence: 0.93051195

00:43:51.370 --> 00:43:53.505 and you can see really beautiful

NOTE Confidence: 0.93051195

00:43:53.505 --> 00:43:54.889 relationships between the two.

NOTE Confidence: 0.93051195

00:43:54.890 --> 00:43:57.270 Any lesion that's V ref P600E wild

NOTE Confidence: 0.93051195

00:43:57.270 --> 00:43:59.250 type also doesn't have brass mutation,

NOTE Confidence: 0.93051195

00:43:59.250 --> 00:44:01.896 almost never has V reps or never

NOTE Confidence: 0.93051195

00:44:01.896 --> 00:44:05.370 has V RP1 phosphorylation positive.

NOTE Confidence: 0.93051195

00:44:05.370 --> 00:44:07.988 And the majority of tumors that are

NOTE Confidence: 0.93051195

00:44:07.988 --> 00:44:10.075 B reference 600D positive always

NOTE Confidence: 0.93051195

00:44:10.075 --> 00:44:12.726 have or approximately 70% of them

NOTE Confidence: 0.93051195

00:44:12.726 --> 00:44:14.458 have D RP1616 positivity.

NOTE Confidence: 0.93051195

00:44:14.460 --> 00:44:17.065 I'm going to visit this again in a

NOTE Confidence: 0.93051195

00:44:17.065 --> 00:44:18.354 few minutes because this doesn't tell

NOTE Confidence: 0.93051195

00:44:18.354 --> 00:44:19.887 you so much information because I mean

NOTE Confidence: 0.93051195

00:44:19.930 --> 00:44:21.338 you can just screen and you can say,

NOTE Confidence: 0.93051195

00:44:21.340 --> 00:44:22.576 well this person has a tumor,

NOTE Confidence: 0.93051195

00:44:22.580 --> 00:44:23.860 it's B roughly 600 positive,

NOTE Confidence: 0.93051195

00:44:23.860 --> 00:44:24.540 they already have a tumor.

NOTE Confidence: 0.93051195

00:44:24.540 --> 00:44:25.600 It's not that interesting,

NOTE Confidence: 0.93051195

00:44:25.600 --> 00:44:27.526 but just kind of a proof of

NOTE Confidence: 0.93051195

00:44:27.526 --> 00:44:29.218 principle that the two are related.

NOTE Confidence: 0.93051195

00:44:29.220 --> 00:44:33.854 So here we're now going into Malana

NOTE Confidence: 0.93051195

00:44:33.854 --> 00:44:37.178 Sites again looking at B ref,

NOTE Confidence: 0.93051195

00:44:37.180 --> 00:44:38.395 P600E and P10.

NOTE Confidence: 0.93051195

00:44:38.395 --> 00:44:40.311 You can of course create,

NOTE Confidence: 0.93051195

00:44:40.311 --> 00:44:42.057 transform Milan of sites and you

NOTE Confidence: 0.93051195

00:44:42.057 --> 00:44:44.336 can see this really nice change and

NOTE Confidence: 0.93051195

00:44:44.336 --> 00:44:46.016 might have come to architecture.

NOTE Confidence: 0.93051195

00:44:46.020 --> 00:44:49.660 And if you eliminate D RP1 in

NOTE Confidence: 0.93051195

00:44:49.660 --> 00:44:52.200 Milan of sites and transform,

NOTE Confidence: 0.93051195

00:44:52.200 --> 00:44:55.880 you now lose the ability for B ref

NOTE Confidence: 0.93051195

00:44:55.880 --> 00:44:58.100 and P10 to transform these steps.

NOTE Confidence: 0.93051195

00:44:58.100 --> 00:44:59.178 What do you think they do instead?

NOTE Confidence: 0.937827649999999

00:45:01.560 --> 00:45:02.008 Undergo senescence.

NOTE Confidence: 0.937827649999999

00:45:02.008 --> 00:45:03.800 But I'm not going to talk about it

NOTE Confidence: 0.937827649999999

00:45:03.849 --> 00:45:05.343 here in the first molecular cell

NOTE Confidence: 0.937827649999999

00:45:05.343 --> 00:45:06.628 paper showed the senescence pathway

NOTE Confidence: 0.937827649999999

00:45:06.628 --> 00:45:08.074 being activated in meths and now

NOTE Confidence: 0.937827649999999

00:45:08.074 --> 00:45:10.220 we're showing the same thing here in

NOTE Confidence: 0.937827649999999

00:45:10.220 --> 00:45:11.995 the in the melanocyte populations.

NOTE Confidence: 0.937827649999999

00:45:12.000 --> 00:45:14.440 So you have to be able to chronically

NOTE Confidence: 0.937827649999999

00:45:14.440 --> 00:45:16.909 divide your mitocundial network in order

NOTE Confidence: 0.937827649999999

00:45:16.909 --> 00:45:19.154 to generate these transformed cells.

NOTE Confidence: 0.937827649999999

00:45:19.160 --> 00:45:21.038 Seems to be established in meths,

NOTE Confidence: 0.937827649999999

00:45:21.040 --> 00:45:21.622 in melanocytes.

NOTE Confidence: 0.937827649999999

00:45:21.622 --> 00:45:23.659 And other people have now done work

NOTE Confidence: 0.937827649999999

00:45:23.659 --> 00:45:26.002 in the pancreas and in the lung in

NOTE Confidence: 0.937827649999999

00:45:26.002 --> 00:45:27.306 different brain tumor initiating

NOTE Confidence: 0.937827649999999

00:45:27.306 --> 00:45:29.236 cells showing the same pathways.

NOTE Confidence: 0.937827649999999

00:45:29.240 --> 00:45:32.488 And what we decided to do was to

NOTE Confidence: 0.937827649999999

00:45:32.488 --> 00:45:35.780 take Melano sites CRISPR 616 A,

NOTE Confidence: 0.937827649999999

00:45:35.780 --> 00:45:37.796 which is basically a mutant form of

NOTE Confidence: 0.937827649999999

00:45:37.796 --> 00:45:40.299 D RP1 that can't divide the network.

NOTE Confidence: 0.937827649999999

00:45:40.300 --> 00:45:42.900 And then we compared it to a CRISPR

NOTE Confidence: 0.937827649999999

00:45:42.900 --> 00:45:44.860 version of 6/16 D chronically

NOTE Confidence: 0.937827649999999

00:45:44.860 --> 00:45:46.096 activated D RP1.

NOTE Confidence: 0.937827649999999

00:45:46.100 --> 00:45:47.132 And you can see what happens

NOTE Confidence: 0.937827649999999

00:45:47.132 --> 00:45:47.820 to these Melano sites.

NOTE Confidence: 0.937827649999999

00:45:47.820 --> 00:45:49.717 I mean they have crazy mitochondria in

NOTE Confidence: 0.937827649999999

00:45:49.717 --> 00:45:51.268 the middle, right? Proof of principle.

NOTE Confidence: 0.937827649999999

00:45:51.268 --> 00:45:52.528 If you can't divide your

NOTE Confidence: 0.937827649999999

00:45:52.528 --> 00:45:53.892 mitochondria network, what happens?

NOTE Confidence: 0.937827649999999

00:45:53.892 --> 00:45:54.644 This might.

NOTE Confidence: 0.937827649999999

00:45:54.644 --> 00:45:57.588 This Melano site just expands up like

NOTE Confidence: 0.937827649999999

00:45:57.588 --> 00:46:00.760 crazy and makes lots of medic country.

NOTE Confidence: 0.937827649999999

00:46:00.760 --> 00:46:02.284 And here you're just simply looking

NOTE Confidence: 0.937827649999999

00:46:02.284 --> 00:46:04.024 at the ability to transform these

NOTE Confidence: 0.937827649999999

00:46:04.024 --> 00:46:05.640 cells can't divide your network,

NOTE Confidence: 0.937827649999999

00:46:05.640 --> 00:46:07.240 You'll never transform these cells,

NOTE Confidence: 0.937827649999999

00:46:07.240 --> 00:46:08.600 and if you're already divided,

NOTE Confidence: 0.937827649999999

00:46:08.600 --> 00:46:09.960 you transform more easily.

NOTE Confidence: 0.92775065

00:46:12.640 --> 00:46:14.117 So this is a really fun experiment

NOTE Confidence: 0.92775065

00:46:14.117 --> 00:46:15.437 and I had to convince people

NOTE Confidence: 0.92775065

00:46:15.437 --> 00:46:16.914 to do this and I love it.

NOTE Confidence: 0.92775065

00:46:16.920 --> 00:46:21.444 So what we can do instead is you can

NOTE Confidence: 0.92775065

00:46:21.444 --> 00:46:22.993 take the line of sites and you can of

NOTE Confidence: 0.92775065

00:46:22.993 --> 00:46:24.477 course introduce an awkward unit to them.

NOTE Confidence: 0.92775065

00:46:24.480 --> 00:46:26.559 And you can activate here at the

NOTE Confidence: 0.92775065

00:46:26.560 --> 00:46:27.920 600B to generate senescent lesions.

NOTE Confidence: 0.9356432

00:46:30.090 --> 00:46:32.880 What I wanted to do is put in a

NOTE Confidence: 0.9356432

00:46:32.880 --> 00:46:33.970 pharmacologically activated form

NOTE Confidence: 0.9356432

00:46:33.970 --> 00:46:36.994 of D RP1 where we can induce

NOTE Confidence: 0.9356432

00:46:36.994 --> 00:46:38.770 its activity with doxycycline.

NOTE Confidence: 0.9356432

00:46:38.770 --> 00:46:40.746 And here what we're using is the 616

NOTE Confidence: 0.9356432

00:46:40.746 --> 00:46:42.928 D So this is the moment it's on,

NOTE Confidence: 0.9356432

00:46:42.930 --> 00:46:44.882 it divides the network.

NOTE Confidence: 0.9356432

00:46:44.882 --> 00:46:49.570 OK And what we did was we took D

NOTE Confidence: 0.9356432

00:46:49.570 --> 00:46:51.715 RP600E generated senescent and lab

NOTE Confidence: 0.9356432

00:46:51.715 --> 00:46:54.410 sites living culture for two months,

NOTE Confidence: 0.9356432

00:46:54.410 --> 00:46:56.858 completely senescent population.

NOTE Confidence: 0.9356432

00:46:56.860 --> 00:47:00.290 And the D RP1 which is pharmacologically

NOTE Confidence: 0.9356432

00:47:00.290 --> 00:47:01.520 activatable selected for,

NOTE Confidence: 0.9356432

00:47:01.520 --> 00:47:03.740 but we never turned it on,

NOTE Confidence: 0.9356432

00:47:03.740 --> 00:47:05.516 but we did instead was once we knew

NOTE Confidence: 0.9356432

00:47:05.516 --> 00:47:07.380 that the populations were senescent,

NOTE Confidence: 0.9356432

00:47:07.380 --> 00:47:10.180 we turn it on and guess what happens,

NOTE Confidence: 0.9356432

00:47:10.180 --> 00:47:12.232 a subset of these cells now

NOTE Confidence: 0.9356432

00:47:12.232 --> 00:47:13.258 reenter cell cycle,

NOTE Confidence: 0.9356432

00:47:13.260 --> 00:47:14.876 divide their mitochondrial network

NOTE Confidence: 0.9356432

00:47:14.876 --> 00:47:16.896 and escape the senescence program.

NOTE Confidence: 0.9356432

00:47:16.900 --> 00:47:19.470 So simply dividing your mitochondrial

NOTE Confidence: 0.9356432

00:47:19.470 --> 00:47:22.040 network is sufficient to drive

NOTE Confidence: 0.9356432

00:47:22.123 --> 00:47:24.218 oncogene into senescence escape.

NOTE Confidence: 0.9356432

00:47:24.218 --> 00:47:25.052 But I mean,

NOTE Confidence: 0.9356432

00:47:25.052 --> 00:47:27.120 who's got the system in their cells?

NOTE Confidence: 0.9356432

00:47:27.120 --> 00:47:30.638 All right, it's not important, it's just fun.

NOTE Confidence: 0.9356432

00:47:30.640 --> 00:47:34.168 What is relevant is if you start

NOTE Confidence: 0.9356432

00:47:34.168 --> 00:47:37.579 to look at what could these

NOTE Confidence: 0.9356432

00:47:37.579 --> 00:47:39.478 cells potentially encounter,

NOTE Confidence: 0.9356432

00:47:39.480 --> 00:47:43.520 Heavy metals, Nicotine.

NOTE Confidence: 0.9356432

00:47:43.520 --> 00:47:44.840 Electronic cigarette residues.

NOTE Confidence: 0.9356432

00:47:44.840 --> 00:47:47.480 The things I'm showing you here

NOTE Confidence: 0.9356432

00:47:47.480 --> 00:47:50.048 and if you make these senescent

NOTE Confidence: 0.9356432

00:47:50.048 --> 00:47:52.690 melanocytes with B raffy 600 E

NOTE Confidence: 0.9356432

00:47:52.690 --> 00:47:54.730 and now you culture them once

NOTE Confidence: 0.9356432

00:47:54.730 --> 00:47:56.090 you know they're senescence.

NOTE Confidence: 0.9356432

00:47:56.090 --> 00:47:56.903 And there were,

NOTE Confidence: 0.9356432

00:47:56.903 --> 00:47:58.529 I should mention the reason why

NOTE Confidence: 0.9356432

00:47:58.529 --> 00:48:00.330 we selected these two reasons.

NOTE Confidence: 0.9356432

00:48:00.330 --> 00:48:03.130 Funding #2.

NOTE Confidence: 0.9356432

00:48:03.130 --> 00:48:06.080 They're all published to make

NOTE Confidence: 0.9356432

00:48:06.080 --> 00:48:07.850 mitochondrial shape changes.

NOTE Confidence: 0.9356432

00:48:07.850 --> 00:48:10.573 You take the cells and you expose

NOTE Confidence: 0.9356432

00:48:10.573 --> 00:48:13.191 them to cadmium chloride or nicotine

NOTE Confidence: 0.9356432

00:48:13.191 --> 00:48:15.834 or .25 puffs per mil of cell culture

NOTE Confidence: 0.9356432

00:48:15.834 --> 00:48:17.890 media of electronic cigarette residue.

NOTE Confidence: 0.9356432

00:48:17.890 --> 00:48:20.150 Banana flavor is the one.

NOTE Confidence: 0.9356432

00:48:20.150 --> 00:48:23.280 It will induce medicantial division and

NOTE Confidence: 0.9356432

00:48:23.280 --> 00:48:26.150 you'll start to get escape from Hawaiias.

NOTE Confidence: 0.9356432

00:48:26.150 --> 00:48:29.486 So you can envision riverbeds

NOTE Confidence: 0.9356432

00:48:29.486 --> 00:48:31.814 that have contaminations,

NOTE Confidence: 0.9356432

00:48:31.814 --> 00:48:32.590 populations,

NOTE Confidence: 0.9356432

00:48:32.590 --> 00:48:34.462 people where they live along rivers

NOTE Confidence: 0.9356432

00:48:34.462 --> 00:48:36.569 and and bodies of water which

NOTE Confidence: 0.9356432

00:48:36.569 --> 00:48:38.549 historically have been quite clean.

NOTE Confidence: 0.9356432

00:48:38.550 --> 00:48:42.070 Places like the Ganga River,

NOTE Confidence: 0.9356432

00:48:42.070 --> 00:48:43.855 different places where there's lots

NOTE Confidence: 0.9356432

00:48:43.855 --> 00:48:46.031 of industry flowing into these rivers

NOTE Confidence: 0.9356432

00:48:46.031 --> 00:48:48.041 and we're starting to see increases

NOTE Confidence: 0.9356432

00:48:48.041 --> 00:48:50.080 in Melanoma genesis along populations.

NOTE Confidence: 0.9356432

00:48:50.080 --> 00:48:51.445 This is environmental toxicity and

NOTE Confidence: 0.9356432

00:48:51.445 --> 00:48:53.166 all of these things by themselves

NOTE Confidence: 0.9356432

00:48:53.166 --> 00:48:54.676 never transform cells and you're

NOTE Confidence: 0.9356432

00:48:54.676 --> 00:48:56.252 never going to take cadmium

NOTE Confidence: 0.9356432

00:48:56.252 --> 00:48:57.516 chloride and do anything.

NOTE Confidence: 0.9356432

00:48:57.520 --> 00:48:59.070 But if you do it in the presence of an

NOTE Confidence: 0.9356432

00:48:59.121 --> 00:49:00.675 Aqua genus cell that's already got it,

NOTE Confidence: 0.9356432

00:49:00.680 --> 00:49:02.437 then you could potentially make an impact.

NOTE Confidence: 0.93842006

00:49:04.680 --> 00:49:06.100 All right. And then I'll

NOTE Confidence: 0.93842006

00:49:06.100 --> 00:49:07.520 be wrapped up very soon.

NOTE Confidence: 0.93842006

00:49:07.520 --> 00:49:09.360 I know we're almost ending.

NOTE Confidence: 0.93842006

00:49:09.360 --> 00:49:12.552 If you look at some of the consequences

NOTE Confidence: 0.93842006

00:49:12.552 --> 00:49:13.813 of transforming melanocytes

NOTE Confidence: 0.93842006

00:49:13.813 --> 00:49:16.208 plus or minus D RP1, you know,

NOTE Confidence: 0.93842006

00:49:16.208 --> 00:49:17.353 here's looking at complex one

NOTE Confidence: 0.93842006

00:49:17.353 --> 00:49:18.745 activity or complex 2 activity and

NOTE Confidence: 0.93842006

00:49:18.745 --> 00:49:20.065 you'll see the complex one activity.

NOTE Confidence: 0.93842006

00:49:20.070 --> 00:49:23.346 When you transform, A lanocyte drops,

NOTE Confidence: 0.93842006

00:49:23.350 --> 00:49:26.895 and it doesn't drop if you

NOTE Confidence: 0.93842006

00:49:26.895 --> 00:49:28.215 inhibit D RP1 beforehand.

NOTE Confidence: 0.93842006

00:49:28.215 --> 00:49:30.015 So this tells us that if you can't

NOTE Confidence: 0.93842006

00:49:30.015 --> 00:49:31.429 divide your multicultural network,

NOTE Confidence: 0.93842006

00:49:31.430 --> 00:49:33.070 you'll never change the

NOTE Confidence: 0.93842006

00:49:33.070 --> 00:49:34.710 metabolism of these cells.

NOTE Confidence: 0.93842006

00:49:34.710 --> 00:49:35.766 And is it important?

NOTE Confidence: 0.93842006

00:49:35.766 --> 00:49:37.350 And this is the experiment that

NOTE Confidence: 0.93842006

00:49:37.410 --> 00:49:38.760 tells you that it's actually

NOTE Confidence: 0.93842006

00:49:38.760 --> 00:49:40.990 important and we can use ND I-1.

NOTE Confidence: 0.93842006

00:49:40.990 --> 00:49:43.190 You know, humans are efficient

NOTE Confidence: 0.93842006

00:49:43.190 --> 00:49:45.429 animals in the most part.

NOTE Confidence: 0.93842006

00:49:45.430 --> 00:49:45.950 You know,

NOTE Confidence: 0.93842006

00:49:45.950 --> 00:49:47.770 our out of our complex one protein

NOTE Confidence: 0.93842006

00:49:47.770 --> 00:49:49.699 is our complex one structure is

NOTE Confidence: 0.93842006

00:49:49.699 --> 00:49:51.304 about 45 proteins and another

NOTE Confidence: 0.93842006

00:49:51.368 --> 00:49:53.198 hundred 150 factors that regulate

NOTE Confidence: 0.93842006

00:49:53.198 --> 00:49:55.028 the assembly and vocalization of

NOTE Confidence: 0.93842006

00:49:55.030 --> 00:49:57.150 this complex yeasts are amazing.

NOTE Confidence: 0.93842006

00:49:57.150 --> 00:49:58.670 They only have one protein

NOTE Confidence: 0.93842006

00:49:58.670 --> 00:50:00.190 that replaces all of this.

NOTE Confidence: 0.93842006

00:50:00.190 --> 00:50:01.710 It is called ND I-1.

NOTE Confidence: 0.93842006

00:50:01.710 --> 00:50:03.467 And if you express it in melanocytes,

NOTE Confidence: 0.93842006

00:50:03.470 --> 00:50:05.420 you get of course increased

NOTE Confidence: 0.93842006

00:50:05.420 --> 00:50:06.590 complex one activity.

NOTE Confidence: 0.93842006

00:50:06.590 --> 00:50:08.366 But what's amazing is if you

NOTE Confidence: 0.93842006

00:50:08.366 --> 00:50:09.550 sustain complex one activity,

NOTE Confidence: 0.93842006

00:50:09.550 --> 00:50:11.190 when you try to transform,

NOTE Confidence: 0.93842006

00:50:11.190 --> 00:50:12.510 you lose the transformation

NOTE Confidence: 0.93842006

00:50:12.510 --> 00:50:13.830 potential of these cells.

NOTE Confidence: 0.93842006

00:50:13.830 --> 00:50:15.710 So they have the graph,

NOTE Confidence: 0.93842006

00:50:15.710 --> 00:50:17.950 they silence tumor suppressor gene,

NOTE Confidence: 0.93842006

00:50:17.950 --> 00:50:20.030 they're dividing the mitochondrial network,

NOTE Confidence: 0.93842006

00:50:20.030 --> 00:50:22.100 but now they're not losing complex

NOTE Confidence: 0.93842006

00:50:22.100 --> 00:50:25.390 one activity and they don't transform.

NOTE Confidence: 0.93842006

00:50:25.390 --> 00:50:27.586 And if we generated a series,

NOTE Confidence: 0.93842006

00:50:27.590 --> 00:50:27.883 oh,

NOTE Confidence: 0.93842006

00:50:27.883 --> 00:50:29.750 I skipped all the data and

NOTE Confidence: 0.93842006

00:50:29.750 --> 00:50:31.750 good luck getting it back.

NOTE Confidence: 0.93842006

00:50:31.750 --> 00:50:33.086 If you look at the reason why when

NOTE Confidence: 0.93842006

00:50:33.086 --> 00:50:34.469 you divide your mitochondrial network,

NOTE Confidence: 0.93842006

00:50:34.470 --> 00:50:35.934 you select for all of these

NOTE Confidence: 0.93842006

00:50:35.934 --> 00:50:37.175 mutations in the mitochondrial gene

NOTE Confidence: 0.93842006

00:50:37.175 --> 00:50:38.507 and that encode for complex one,

NOTE Confidence: 0.93842006

00:50:38.510 --> 00:50:39.750 all right,

NOTE Confidence: 0.93842006

00:50:39.750 --> 00:50:41.310 Kind of a proof of principle

NOTE Confidence: 0.93842006

00:50:41.310 --> 00:50:43.470 of that is we take melanocytes,

NOTE Confidence: 0.93842006

00:50:43.470 --> 00:50:44.748 and you know when you do,

NOTE Confidence: 0.93842006

00:50:44.750 --> 00:50:46.106 when you transform melanocytes,

NOTE Confidence: 0.93842006

00:50:46.106 --> 00:50:47.801 you get a heterogeneous population

NOTE Confidence: 0.93842006

00:50:47.801 --> 00:50:49.269 of mitochondrial genomes,

NOTE Confidence: 0.93842006

00:50:49.270 --> 00:50:50.366 mutations throughout the genome.

NOTE Confidence: 0.93842006

00:50:50.366 --> 00:50:51.865 Some are important, some are not.

NOTE Confidence: 0.93842006

00:50:51.865 --> 00:50:54.429 So what we do is we subject them to ethidium,

NOTE Confidence: 0.93842006

00:50:54.430 --> 00:50:57.710 bromide, pyruvate and uranine selection.

NOTE Confidence: 0.93842006

00:50:57.710 --> 00:50:59.414 That allows us to basically kick

NOTE Confidence: 0.93842006

00:50:59.414 --> 00:51:01.024 out all of the mitochondrial

NOTE Confidence: 0.93842006

00:51:01.024 --> 00:51:03.534 genomes in that cell and we reduce

NOTE Confidence: 0.93842006

00:51:03.534 --> 00:51:05.262 it to 1 genome per cell.

NOTE Confidence: 0.93842006

00:51:05.270 --> 00:51:07.190 You take the pressure off and that

NOTE Confidence: 0.93842006

00:51:07.190 --> 00:51:08.415 one genome repopulates the cells.

NOTE Confidence: 0.93842006

00:51:08.420 --> 00:51:11.060 So now you create these clonal

NOTE Confidence: 0.93842006

00:51:11.060 --> 00:51:12.820 melanocytes that are transformed.

NOTE Confidence: 0.93842006

00:51:12.820 --> 00:51:16.272 You implant them into animals and

NOTE Confidence: 0.93842006

00:51:16.272 --> 00:51:18.876 what you see is controlled cells of

NOTE Confidence: 0.93842006

00:51:18.876 --> 00:51:21.740 course have normal complex one activity,

NOTE Confidence: 0.93842006

00:51:21.740 --> 00:51:22.940 no tumor volume.

NOTE Confidence: 0.93842006

00:51:22.940 --> 00:51:23.740 You transform,

NOTE Confidence: 0.93842006

00:51:23.740 --> 00:51:25.180 they lose complex one activity.

NOTE Confidence: 0.93842006

00:51:25.180 --> 00:51:26.380 I really showed you that.

NOTE Confidence: 0.93842006

00:51:26.380 --> 00:51:28.494 And they generate A tumor and growth.

NOTE Confidence: 0.93842006

00:51:28.500 --> 00:51:30.607 Zero cells have no complex one activity

NOTE Confidence: 0.93842006

00:51:30.607 --> 00:51:32.290 of course the Pentium mitochondrial

NOTE Confidence: 0.93842006

00:51:32.290 --> 00:51:34.810 genome and they don't form a tumor.

NOTE Confidence: 0.93842006

00:51:34.810 --> 00:51:37.946 Row 0 is this process of eliminating

NOTE Confidence: 0.93842006

00:51:37.946 --> 00:51:39.290 the mitochondrial genome.

NOTE Confidence: 0.93842006

00:51:39.290 --> 00:51:41.600 You take 10 clones that have

NOTE Confidence: 0.93842006

00:51:41.600 --> 00:51:44.050 decreasing ability to have complex one,

NOTE Confidence: 0.93842006

00:51:44.050 --> 00:51:47.082 which you see is this sweet spot of

NOTE Confidence: 0.93842006

00:51:47.082 --> 00:51:49.260 tumor formation occurring in this 30

NOTE Confidence: 0.93842006

00:51:49.260 --> 00:51:53.130 to 40% deletion of of complex one.

NOTE Confidence: 0.93842006

00:51:53.130 --> 00:51:54.792 And this exactly mirrors the levels

NOTE Confidence: 0.93842006

00:51:54.792 --> 00:51:57.078 of complex one that you see when you

NOTE Confidence: 0.93842006

00:51:57.078 --> 00:51:58.770 transform these these cells in culture.

NOTE Confidence: 0.9282218

00:52:01.390 --> 00:52:02.678 Small mitochondria are bad.

NOTE Confidence: 0.9282218

00:52:02.678 --> 00:52:04.610 Another reason why they're bad is

NOTE Confidence: 0.9282218

00:52:04.671 --> 00:52:06.449 paper that we published a long time

NOTE Confidence: 0.9282218

00:52:06.449 --> 00:52:08.584 ago was it's really hard to kill

NOTE Confidence: 0.9282218

00:52:08.584 --> 00:52:09.904 cells with small mitochondria.

NOTE Confidence: 0.9282218

00:52:09.910 --> 00:52:11.382 The reason why we got on to this

NOTE Confidence: 0.9282218

00:52:11.382 --> 00:52:12.828 is in the cell death field,

NOTE Confidence: 0.9282218

00:52:12.830 --> 00:52:14.430 it's knowing that if cells

NOTE Confidence: 0.9282218

00:52:14.430 --> 00:52:15.390 are undergoing mitosis,

NOTE Confidence: 0.9282218

00:52:15.390 --> 00:52:17.640 you can't induce mitochondria out

NOTE Confidence: 0.9282218

00:52:17.640 --> 00:52:19.890 of membrane reverbalization of that

NOTE Confidence: 0.9282218

00:52:19.963 --> 00:52:21.918 process of releasing cytochrome C

NOTE Confidence: 0.9282218

00:52:21.918 --> 00:52:24.470 to activate the cell death pathway.

NOTE Confidence: 0.9282218

00:52:24.470 --> 00:52:27.728 And you also whenever you have

NOTE Confidence: 0.9282218

00:52:27.728 --> 00:52:29.357 hyperframeants of mitochondria,

NOTE Confidence: 0.9282218

00:52:29.360 --> 00:52:30.935 it's really complicated to treat

NOTE Confidence: 0.9282218

00:52:30.935 --> 00:52:31.880 them with chemo,

NOTE Confidence: 0.9282218

00:52:31.880 --> 00:52:33.280 the the cell lines themselves,

NOTE Confidence: 0.9282218

00:52:33.280 --> 00:52:34.678 they just don't respond very well.

NOTE Confidence: 0.9282218

00:52:34.680 --> 00:52:36.276 And we figured out the mechanism here,

NOTE Confidence: 0.9282218

00:52:36.280 --> 00:52:37.592 basically the Bax protein,

NOTE Confidence: 0.9282218

00:52:37.592 --> 00:52:39.560 everybody knows the BCL two family

NOTE Confidence: 0.9282218

00:52:39.621 --> 00:52:41.397 with Doctor Katz in the audience.

NOTE Confidence: 0.9282218

00:52:41.400 --> 00:52:43.196 These provototic proteins can't

NOTE Confidence: 0.9282218

00:52:43.196 --> 00:52:45.441 link into the outer mitochondria

NOTE Confidence: 0.9282218

00:52:45.441 --> 00:52:46.998 membrane to drive them up.

NOTE Confidence: 0.9282218

00:52:47.000 --> 00:52:47.880 And furthermore,

NOTE Confidence: 0.9282218

00:52:47.880 --> 00:52:50.080 when you have many mitochondria

NOTE Confidence: 0.9282218

00:52:50.080 --> 00:52:52.835 in the cell and you have a fixed

NOTE Confidence: 0.9282218

00:52:52.835 --> 00:52:54.840 repertoire of provototic BCL 2 proteins,

NOTE Confidence: 0.9282218

00:52:54.840 --> 00:52:57.360 it's also harder to drive cytochrome

NOTE Confidence: 0.9282218

00:52:57.360 --> 00:52:58.620 cereals and apoptosis.

NOTE Confidence: 0.9282218

00:52:58.620 --> 00:52:59.322 Mindy, you know,

NOTE Confidence: 0.9282218

00:52:59.322 --> 00:53:00.726 this is a really beautiful paper

NOTE Confidence: 0.9282218

00:53:00.726 --> 00:53:02.028 that basically showed the

NOTE Confidence: 0.9282218

00:53:02.028 --> 00:53:03.340 more mitochondria you have,

NOTE Confidence: 0.9282218

00:53:03.340 --> 00:53:04.936 the more mitochondrial mass you have,

NOTE Confidence: 0.9282218

00:53:04.940 --> 00:53:06.974 the more drug it takes to kill the cell.

NOTE Confidence: 0.9282218

00:53:06.980 --> 00:53:07.980 The reason is really simple.

NOTE Confidence: 0.9282218

00:53:07.980 --> 00:53:09.723 If you have 100 molecules of facts

NOTE Confidence: 0.9282218

00:53:09.723 --> 00:53:11.380 and you have 10 mitochondria,

NOTE Confidence: 0.9282218

00:53:11.380 --> 00:53:12.660 every mitochondria gets 10

NOTE Confidence: 0.9282218

00:53:12.660 --> 00:53:14.580 molecules and you create a four.

NOTE Confidence: 0.9282218

00:53:14.580 --> 00:53:16.225 Now you have 100 molecules of facts

NOTE Confidence: 0.9282218

00:53:16.225 --> 00:53:17.819 and you have 100 mitochondria.

NOTE Confidence: 0.9282218

00:53:17.820 --> 00:53:19.662 Each mitochondria gets one molecule of

NOTE Confidence: 0.9282218

00:53:19.662 --> 00:53:21.700 facts and you'll never kill that cell.

NOTE Confidence: 0.9301034

00:53:27.850 --> 00:53:30.554 I think I'm going to end with this

NOTE Confidence: 0.9301034

00:53:30.554 --> 00:53:33.178 slide here where what we decided to

NOTE Confidence: 0.9301034

00:53:33.178 --> 00:53:36.735 do is we use this mouse antibody that

NOTE Confidence: 0.9301034

00:53:36.735 --> 00:53:38.835 detects the phosphorylation event

NOTE Confidence: 0.9301034

00:53:38.835 --> 00:53:42.090 on D RP1 and we screen through many

NOTE Confidence: 0.9301034

00:53:42.090 --> 00:53:43.770 different types of skin lesions.

NOTE Confidence: 0.9301034

00:53:43.770 --> 00:53:46.860 Normal skin never positive dysplastic

NOTE Confidence: 0.9301034

00:53:46.860 --> 00:53:50.350 nevi almost always positive Melanoma in

NOTE Confidence: 0.9301034

00:53:50.350 --> 00:53:53.050 C2 about 70% and then any non Melanoma,

NOTE Confidence: 0.9301034

00:53:53.050 --> 00:53:55.316 any non B ref Melanoma 7%.

NOTE Confidence: 0.9301034

00:53:55.316 --> 00:53:56.846 Again that's not that interesting

NOTE Confidence: 0.9301034

00:53:56.846 --> 00:53:58.710 because you know you're just kind

NOTE Confidence: 0.9301034

00:53:58.710 --> 00:54:00.180 of following the process along.

NOTE Confidence: 0.9301034

00:54:00.180 --> 00:54:01.992 What's curious is you know the

NOTE Confidence: 0.9301034

00:54:01.992 --> 00:54:03.984 vast majority of these lesions are

NOTE Confidence: 0.9301034

00:54:03.984 --> 00:54:05.779 being referenced to be positive,

NOTE Confidence: 0.9301034

00:54:05.780 --> 00:54:07.586 but it doesn't necessarily tell you if

NOTE Confidence: 0.9301034

00:54:07.586 --> 00:54:09.699 you're going to eventually develop disease.

NOTE Confidence: 0.9301034

00:54:09.700 --> 00:54:12.124 So what we did instead was we did

NOTE Confidence: 0.9301034

00:54:12.124 --> 00:54:13.828 something called the discovery cohort

NOTE Confidence: 0.9301034

00:54:13.828 --> 00:54:18.340 where we took 300 and 53150 biopsies

NOTE Confidence: 0.9301034

00:54:18.340 --> 00:54:22.600 and within those 350 biopsies were

NOTE Confidence: 0.9301034

00:54:22.600 --> 00:54:26.100 35 patients that developed disease.

NOTE Confidence: 0.9301034

00:54:26.100 --> 00:54:28.156 What we're able to do is we could

NOTE Confidence: 0.9301034

00:54:28.156 --> 00:54:30.256 screen with the D RP1 antibody and we

NOTE Confidence: 0.9301034

00:54:30.256 --> 00:54:32.980 could pull out 31 of the 35 patients,

NOTE Confidence: 0.9301034

00:54:32.980 --> 00:54:34.636 suggesting that if you know your

NOTE Confidence: 0.9301034

00:54:34.636 --> 00:54:36.658 status of D RP1 in that lesion,

NOTE Confidence: 0.9301034

00:54:36.660 --> 00:54:38.430 it could potentially inform you on

NOTE Confidence: 0.9301034

00:54:38.430 --> 00:54:40.168 the likelihood of getting disease and

NOTE Confidence: 0.9301034

00:54:40.168 --> 00:54:42.176 maybe inform you on the number of times

NOTE Confidence: 0.9301034

00:54:42.228 --> 00:54:44.100 you should get these lesions screened.

NOTE Confidence: 0.9301034

00:54:44.100 --> 00:54:47.900 And if you're super keen in the audience,

NOTE Confidence: 0.9301034

00:54:47.900 --> 00:54:50.238 it creates quite of a really interesting

NOTE Confidence: 0.9301034

00:54:50.238 --> 00:54:51.986 biological question because here the lesion

NOTE Confidence: 0.9301034

00:54:51.986 --> 00:54:54.035 is gone and you screened it for B RAF

NOTE Confidence: 0.9301034

00:54:54.035 --> 00:54:56.674 or you screened it for both B RAF and D RP1.

NOTE Confidence: 0.9301034

00:54:56.674 --> 00:54:59.250 How is it you still develop disease

NOTE Confidence: 0.9301034

00:54:59.330 --> 00:55:00.701 and it you develop disease,

NOTE Confidence: 0.9301034

00:55:00.701 --> 00:55:02.483 but the original lesion is gone,

NOTE Confidence: 0.9301034

00:55:02.490 --> 00:55:03.828 but D RP1 still tells you

NOTE Confidence: 0.9301034

00:55:03.828 --> 00:55:05.129 if you're going to get it.

NOTE Confidence: 0.9301034

00:55:05.130 --> 00:55:06.290 It's a really amazing biology.

NOTE Confidence: 0.9301034

00:55:06.290 --> 00:55:07.837 And I have some ideas in terms

NOTE Confidence: 0.9301034

00:55:07.837 --> 00:55:09.809 of why I think that's the case.

NOTE Confidence: 0.9301034

00:55:09.810 --> 00:55:11.400 We developed a human specific antibody

NOTE Confidence: 0.9301034

00:55:11.400 --> 00:55:12.725 that's recombinant and we're doing

NOTE Confidence: 0.9301034

00:55:12.725 --> 00:55:14.045 that rather than using the mouse.

NOTE Confidence: 0.9301034

00:55:14.050 --> 00:55:15.961 We made one that's say human specific

NOTE Confidence: 0.9301034

00:55:15.961 --> 00:55:17.850 and it also works beautifully.

NOTE Confidence: 0.9301034

00:55:17.850 --> 00:55:22.524 So what I showed you today was a bunch

NOTE Confidence: 0.9301034

00:55:22.524 --> 00:55:25.364 of programs looking at mitochondrial

NOTE Confidence: 0.9301034

00:55:25.364 --> 00:55:27.074 control of linosite biology,

NOTE Confidence: 0.9301034

00:55:27.074 --> 00:55:27.648 Melanoma, genesis.

NOTE Confidence: 0.9301034

00:55:27.648 --> 00:55:29.370 I don't need to read everything

NOTE Confidence: 0.9301034

00:55:29.413 --> 00:55:30.297 that's going on here.

NOTE Confidence: 0.9301034

00:55:30.300 --> 00:55:32.204 This is just a summary of what

NOTE Confidence: 0.9301034

00:55:32.204 --> 00:55:33.020 I've been doing.

NOTE Confidence: 0.9301034

00:55:33.020 --> 00:55:35.996 At the moment we're looking at a TF5

NOTE Confidence: 0.9301034

00:55:35.996 --> 00:55:39.339 in the MiTo UPR in skin development.

NOTE Confidence: 0.9301034

00:55:39.340 --> 00:55:41.468 We're looking at some of the MiTo

NOTE Confidence: 0.9301034

00:55:41.468 --> 00:55:43.579 UPR regulation of spatial metabolism,

NOTE Confidence: 0.9301034

00:55:43.580 --> 00:55:44.608 gene expression,

NOTE Confidence: 0.9301034

00:55:44.608 --> 00:55:47.178 changes in skin and different

NOTE Confidence: 0.9301034

00:55:47.178 --> 00:55:49.310 types of tumor types.

NOTE Confidence: 0.9301034

00:55:49.310 --> 00:55:52.028 And we're of course looking at all of these

NOTE Confidence: 0.9301034

00:55:52.028 --> 00:55:53.765 different ways of affecting this process.

NOTE Confidence: 0.9301034

00:55:53.765 --> 00:55:56.040 And one of the things we're doing

NOTE Confidence: 0.9301034

00:55:56.104 --> 00:55:58.379 is making a proof of concept protact

NOTE Confidence: 0.9301034

00:55:58.379 --> 00:56:00.061 against ATF5 protein because it's

NOTE Confidence: 0.9301034

00:56:00.061 --> 00:56:01.987 really a hard protein to target.

NOTE Confidence: 0.9301034

00:56:01.990 --> 00:56:04.710 So these are all the people who do the work,

NOTE Confidence: 0.9301034

00:56:04.710 --> 00:56:05.114 collaborators,

NOTE Confidence: 0.9301034

00:56:05.114 --> 00:56:05.922 external collaborators,

NOTE Confidence: 0.9301034

00:56:05.922 --> 00:56:09.142 some of the people who support the group

NOTE Confidence: 0.9301034

00:56:09.142 --> 00:56:11.104 because they provide us with young

NOTE Confidence: 0.9301034

00:56:11.104 --> 00:56:13.528 scientists and the people who pay the bills.

NOTE Confidence: 0.9301034

00:56:13.530 --> 00:56:14.770 And I think for being a great audience.

NOTE Confidence: 0.9301034

00:56:14.770 --> 00:56:16.330 And I saw lots of heads shaking and

NOTE Confidence: 0.9301034

00:56:16.330 --> 00:56:17.809 smiling and stuff when I was talking.

NOTE Confidence: 0.89781743

00:56:17.810 --> 00:56:18.979 And I like that because it means

NOTE Confidence: 0.89781743

00:56:18.979 --> 00:56:20.130 I have people hear me. Thank you.

NOTE Confidence: 0.89781743

00:56:31.970 --> 00:56:32.210 Hi.

NOTE Confidence: 0.9283398

00:56:42.270 --> 00:56:45.210 Yeah, it is the classic and

NOTE Confidence: 0.9283398

00:56:45.210 --> 00:56:47.650 I think if anyone knows me,

NOTE Confidence: 0.9283398

00:56:47.650 --> 00:56:51.790 I'm not classic and so I'm traditional.

NOTE Confidence: 0.9283398

00:56:51.790 --> 00:56:54.078 We haven't done anything yet and that's where

NOTE Confidence: 0.9283398

00:56:54.078 --> 00:56:56.590 I want to start with the UV in Lenosites.

NOTE Confidence: 0.9283398

00:56:56.590 --> 00:56:58.270 I want to use things like the Yummers,

NOTE Confidence: 0.9283398

00:56:58.270 --> 00:56:59.509 like all of these types of models.

NOTE Confidence: 0.9283398

00:56:59.510 --> 00:57:00.986 We just haven't gone there yet.

NOTE Confidence: 0.9283398

00:57:00.990 --> 00:57:03.482 But this the, the movement from normal

NOTE Confidence: 0.9283398

00:57:03.482 --> 00:57:05.950 to the senescence and those phenotypes,

NOTE Confidence: 0.9283398

00:57:05.950 --> 00:57:08.698 all of this data are fresh.

NOTE Confidence: 0.9283398

00:57:08.700 --> 00:57:10.932 And you'll see some data are like really nice

NOTE Confidence: 0.9283398

00:57:10.932 --> 00:57:13.175 blue and red and some other data are pastel.

NOTE Confidence: 0.9283398

00:57:13.180 --> 00:57:14.321 The pastel is the good data and

NOTE Confidence: 0.9283398

00:57:14.321 --> 00:57:15.679 the blue and red is the stuff

NOTE Confidence: 0.9283398

00:57:15.679 --> 00:57:16.725 that's being replaced, right.

NOTE Confidence: 0.9283398

00:57:16.725 --> 00:57:19.700 So it's, it's active by your question.

NOTE Confidence: 0.9283398

00:57:19.700 --> 00:57:23.140 Yes, we will. I don't have a Gray,

NOTE Confidence: 0.9283398

00:57:23.140 --> 00:57:25.388 you know, telenova program at Sinai.

NOTE Confidence: 0.9283398

00:57:25.388 --> 00:57:26.174 It's not huge.

NOTE Confidence: 0.9283398

00:57:26.180 --> 00:57:29.576 It's like 2 investigators and this is like

NOTE Confidence: 0.92699605

00:57:47.080 --> 00:57:48.376 the experiment. Yeah,

NOTE Confidence: 0.92699605

00:57:48.376 --> 00:57:51.400 this is we don't have that information.

NOTE Confidence: 0.92699605

00:57:51.400 --> 00:57:53.304 I always wonder how you can take

NOTE Confidence: 0.92699605

00:57:53.304 --> 00:57:55.413 you know these are just these are

NOTE Confidence: 0.92699605

00:57:55.413 --> 00:57:57.255 nations that were taken off biopsy

NOTE Confidence: 0.92699605

00:57:57.319 --> 00:57:59.175 and then we score for D RP1 and

NOTE Confidence: 0.92699605

00:57:59.175 --> 00:58:02.902 tell the language that's about most

NOTE Confidence: 0.92699605

00:58:02.902 --> 00:58:06.136 of the time at a different size.

NOTE Confidence: 0.92699605

00:58:06.140 --> 00:58:08.180 We don't have a complete data set there.

NOTE Confidence: 0.92699605

00:58:08.180 --> 00:58:10.730 How molds are communicating with each

NOTE Confidence: 0.92699605

00:58:10.730 --> 00:58:13.490 other about me by are communicating with

NOTE Confidence: 0.92699605

00:58:13.490 --> 00:58:16.435 each other I predict will of course

NOTE Confidence: 0.92699605

00:58:16.435 --> 00:58:20.200 be to be soluble factors like and I

NOTE Confidence: 0.92699605

00:58:20.200 --> 00:58:22.590 predict that my world is all about

NOTE Confidence: 0.92699605

00:58:22.590 --> 00:58:25.292 what I study So you know understanding

NOTE Confidence: 0.92699605

00:58:25.292 --> 00:58:27.244 how mitocrontial division informs

NOTE Confidence: 0.92699605

00:58:27.244 --> 00:58:29.382 mitocrantial quality control which then

NOTE Confidence: 0.92699605

00:58:29.382 --> 00:58:31.578 impacts on how these cells secrete.

NOTE Confidence: 0.92699605

00:58:31.580 --> 00:58:35.210 We already know that mitocrantry controls.

NOTE Confidence: 0.92699605

00:58:35.210 --> 00:58:37.451 I don't think it's a surprise now that you

NOTE Confidence: 0.92699605

00:58:37.451 --> 00:58:39.609 have a major option regulating the form,

NOTE Confidence: 0.92699605

00:58:39.610 --> 00:58:41.932 the composition and the quality control

NOTE Confidence: 0.92699605

00:58:41.932 --> 00:58:44.846 of the organome that you can secrete that

NOTE Confidence: 0.92699605

00:58:44.846 --> 00:58:47.606 will form another place or it's just,

NOTE Confidence: 0.92699605

00:58:47.610 --> 00:58:48.302 you know,

NOTE Confidence: 0.92699605

00:58:48.302 --> 00:58:49.686 disease that already disseminated.

NOTE Confidence: 0.92699605

00:58:49.690 --> 00:58:51.754 We took it off and it just happens

NOTE Confidence: 0.92699605

00:58:51.754 --> 00:58:53.370 to be residual either one.

NOTE Confidence: 0.92699605

00:58:53.370 --> 00:58:54.930 But I still think it's interesting.

NOTE Confidence: 0.92699605

00:58:54.930 --> 00:58:56.914 And if you can take the lesion off

NOTE Confidence: 0.92699605

00:58:56.914 --> 00:58:58.489 and predict who's going to get,

NOTE Confidence: 0.92699605

00:58:58.490 --> 00:59:00.610 it might be a good way of screening.

NOTE Confidence: 0.90922284

00:59:05.700 --> 00:59:05.780 All

NOTE Confidence: 0.90922284

00:59:08.340 --> 00:59:09.180 right. Some

NOTE Confidence: 0.90922284

00:59:13.980 --> 00:59:15.100 trap application.

NOTE Confidence: 0.90922284

00:59:22.140 --> 00:59:22.500 Yeah.

NOTE Confidence: 0.90922284

00:59:26.940 --> 00:59:30.750 Yeah. So, yeah, exactly the question.

NOTE Confidence: 0.90922284

00:59:30.750 --> 00:59:32.748 We don't know. So, you know,

NOTE Confidence: 0.90922284

00:59:32.750 --> 00:59:34.710 we've done the fibroblast work.

NOTE Confidence: 0.90922284

00:59:34.710 --> 00:59:36.935 We've done the melanicite work

NOTE Confidence: 0.90922284

00:59:36.935 --> 00:59:39.530 looking at inducible forms of Ras,

NOTE Confidence: 0.90922284

00:59:39.530 --> 00:59:43.822 inducible forms of H, Ras, G12, the Q1,

NOTE Confidence: 0.90922284

00:59:43.822 --> 00:59:47.958 the Q61 series and it's not clear.

NOTE Confidence: 0.90922284

00:59:47.958 --> 00:59:50.630 So to add a more lack of

NOTE Confidence: 0.90922284

00:59:50.630 --> 00:59:51.750 clarity to the situation,

NOTE Confidence: 0.90922284

00:59:51.750 --> 00:59:54.902 I have a student now who's screening also

NOTE Confidence: 0.90922284

00:59:54.902 --> 00:59:57.010 in Drosophila try to help figure this out.

NOTE Confidence: 0.90922284

00:59:57.010 --> 00:59:58.778 I mean I I assume it's because of

NOTE Confidence: 0.90922284

00:59:58.778 --> 01:00:00.494 the way rats affects multiple arms

NOTE Confidence: 0.90922284

01:00:00.494 --> 01:00:02.636 of the pathway versus just if the

NOTE Confidence: 0.90922284

01:00:02.636 --> 01:00:04.526 rats mutation is a bit more focused.

NOTE Confidence: 0.90922284

01:00:04.530 --> 01:00:05.570 And if that's the case,

NOTE Confidence: 0.90922284

01:00:05.570 --> 01:00:07.145 I'm hoping we can do some of the genetics

NOTE Confidence: 0.90922284

01:00:07.145 --> 01:00:08.568 and your software to figure it out.

NOTE Confidence: 0.90922284

01:00:08.570 --> 01:00:10.342 So we are, you know, we are,

NOTE Confidence: 0.90922284

01:00:10.342 --> 01:00:12.204 we're regulating the might of UPR pathway,

NOTE Confidence: 0.90922284

01:00:12.210 --> 01:00:13.675 we're regulating muddy Contra division

NOTE Confidence: 0.90922284

01:00:13.675 --> 01:00:15.140 machinery there because you can

NOTE Confidence: 0.90922284

01:00:15.187 --> 01:00:16.567 look at developments of the wing,

NOTE Confidence: 0.90922284

01:00:16.570 --> 01:00:17.525 you could look at development

NOTE Confidence: 0.90922284

01:00:17.525 --> 01:00:18.650 of stress signaling in the eye,

NOTE Confidence: 0.90922284

01:00:18.650 --> 01:00:20.085 you can look at a bunch of.

NOTE Confidence: 0.90922284

01:00:20.090 --> 01:00:20.948 Wing development, phenotypes.

NOTE Confidence: 0.90922284

01:00:20.948 --> 01:00:23.306 You can parse out a lot of things

NOTE Confidence: 0.90922284

01:00:23.306 --> 01:00:25.088 because there's such a huge literature

NOTE Confidence: 0.90922284

01:00:25.088 --> 01:00:27.382 there on how each one of those

NOTE Confidence: 0.90922284

01:00:27.382 --> 01:00:28.734 phenotypes is genetically controlled

NOTE Confidence: 0.90922284

01:00:28.734 --> 01:00:31.330 by distinct portions of the pathway.

NOTE Confidence: 0.90922284

01:00:31.330 --> 01:00:32.730 I guess that's the best answer.

NOTE Confidence: 0.90922284

01:00:32.730 --> 01:00:33.432 We don't know,

NOTE Confidence: 0.90922284

01:00:33.432 --> 01:00:35.409 but we're kind of starting to work on it.

NOTE Confidence: 0.90922284

01:00:35.410 --> 01:00:36.964 But maybe not the most elegant thing.

NOTE Confidence: 0.93106353

01:00:45.850 --> 01:00:46.090 Yeah,

NOTE Confidence: 0.9258604

01:00:48.270 --> 01:00:49.734 yeah, minutes, actually.

NOTE Confidence: 0.9258604

01:00:49.734 --> 01:00:52.662 So if you treat with vemurafenib,

NOTE Confidence: 0.9258604

01:00:52.670 --> 01:00:53.810 you can basically put

NOTE Confidence: 0.9258604

01:00:53.810 --> 01:00:54.950 it under the microscope.

NOTE Confidence: 0.9258604

01:00:54.950 --> 01:00:56.266 You can take the plate of cells,

NOTE Confidence: 0.9258604

01:00:56.270 --> 01:00:58.590 treat with vemurafenib, a 370.

NOTE Confidence: 0.9258604

01:00:58.590 --> 01:01:00.870 Fives will be fully fused within two hours.

NOTE Confidence: 0.9258604

01:01:00.870 --> 01:01:02.590 You'll see increases in TMRA.

NOTE Confidence: 0.9258604

01:01:02.590 --> 01:01:06.054 You can do it like flow is super, super fast.

NOTE Confidence: 0.9258604

01:01:06.054 --> 01:01:07.710 They're extremely dynamic,

NOTE Confidence: 0.9258604

01:01:07.710 --> 01:01:08.914 suggesting that mitochondria in

NOTE Confidence: 0.9258604

01:01:08.914 --> 01:01:10.720 these tumors are just kind of

NOTE Confidence: 0.9258604

01:01:10.774 --> 01:01:11.909 primed and ready to go.

NOTE Confidence: 0.9258604

01:01:11.910 --> 01:01:13.945 And you refuse the exchange

NOTE Confidence: 0.9258604

01:01:13.945 --> 01:01:15.166 and they're fixed.

NOTE Confidence: 0.9258604

01:01:15.170 --> 01:01:17.282 So we actually have a really

NOTE Confidence: 0.9258604

01:01:17.282 --> 01:01:19.466 interesting aim and a grant looking

NOTE Confidence: 0.9258604

01:01:19.466 --> 01:01:21.590 at how the diversity of mitochondria

NOTE Confidence: 0.9258604

01:01:21.590 --> 01:01:23.365 within a particular cancer cell,

NOTE Confidence: 0.9258604

01:01:23.370 --> 01:01:26.430 the heterogeneity can be overcome by

NOTE Confidence: 0.9258604

01:01:26.430 --> 01:01:28.650 fusing them and allowing for repair.

NOTE Confidence: 0.925054

01:01:31.810 --> 01:01:33.090 Maybe we'll take the additional

NOTE Confidence: 0.925054

01:01:33.850 --> 01:01:37.010 questions up front. Thank you.