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 We're going to continue our discussion about cancer and cancer genetics today. One small point of clarification: apparently some people were confused about my out of balance scales here related to proliferation and cell death, pointing out that if there were more proliferation and less cell death, then the scale should shift this way instead of this way. That's an astute observation.

It doesn't really matter which way the scales point really.

The point was that they are out of balance, which is critical for tumorigenesis. But anyway, in review, last time I told you that cancer cells arise from normal cells over a series of events that relate to mutations in cellular genes.

And I gave you lots of evidence that things that cause cancer affect our genes, and that our genes are disrupted in cancer cells.

And I briefly mentioned that we have evidence for mutations in cellular genes in cancer cells. And I'll show you that again today.

But all of that brings us to the point of needing to know, what are the genes? What are the genes that are affected in cancer?

And this is important to help us understand how cancers arise, so from a basic biomedical research point of view, but also as we'll see next time, this information is actually very useful in teaching us how to diagnose cancer more effectively or treat it more effectively. And I'll just show this slide, which I showed at the end again as a summary. The cancer cells arise from normal cells through the acquisition of mutations in cellular genes through this process which we call clonal evolution, more and more abnormal cells growing out from this accumulating mass of cells. And as we'll discuss today, these genes affect proliferation and cell death, this out of balance scheme that I alluded to before, but not only these processes.

And I emphasize this because these other important processes get a little bit of short shrift in our discussions. But angiogenesis, this process of recruiting a new blood supply in cancer is very important, and also an important therapeutic target.

Cell motility, movement of cells, as I mentioned, it's important to get cells away from the initial mass in the process of metastasis, and invasion likewise. And several other processes are also affected in the course of tumorigenesis. So what are the genes?

How do we know which genes are affected in cancer?

Well, this set of experiments, really, has occurred over the course of arguably the last century. I'm trying to find the genes that are mutated in cancer cells. And many people point to landmark experiments done in 1910 by an

investigator at the Rockefeller University by the name of Payton Rauss.

Payton Rauss was a virologist, studied viruses at Rockefeller University. And one day, a farmer from Long Island brought a prize-winning hen to this famous doctor at Rockefeller University in New York because the hen was sick. Specifically, the hen had a big tumor in its breast muscle. And if you remember, that would mean the tumor was a sarcoma. And he wanted the doctor to cure his prize-winning hen. Payton Rauss said, thank you, I'll do what I can, and then prominently killed the bird in order to try to study what was giving rise to this tumor.

And he did a famous experiment.

He took the tumor, ground it up, filtered it so that there were no cells present in the filtrate, and also used a small enough pour filter so that bacteria, which were also known at that time, were removed. So, he had a filtrate that contained no cells, no cancer cells, and no bacteria.

And he took this and injected it into a non-tumor bearing bird.

And he observed over time that this bird developed a tumor.

So he was able to demonstrate the transmissibility of something which could cause cancer in an otherwise unaffected animal.

Because it wasn't cells and it wasn't bacteria, he suggested that it was a virus.

And it turned out to be, over the course of the next 50 years or so, the virus which was responsible for this disease was identified and given the name Rauss sarcoma virus, named after Payton Rauss. And the structure of this virus was also determined. It was found to be a retrovirus.

Retroviruses are in the class of HIV.

Their genomes are made of RNA, and they convert it to a DNA form.

We'll learn a little bit more about retroviruses in a few lectures.

It was a retrovirus, and the structure of its genome was determined eventually. And it was found to contain in its genome the genes that the virus required for replication.

And all viruses of this class has a very similar set of replication genes. They need to produce more virus. But at the end of the viral genome, at the extreme right side of the viral genome, there was another gene which was given the name sarc for sarcoma.

And it was termed an oncogene, onco for mass, oncology, cancer associated gene, sarc-oncogene.

So, the virus it was found can cause cancer in birds by virtue of transferring into those cells a potent cancer associated gene called sarc. And Payton Rauss went onto win the Nobel Prize, actually about 50 years later in the early 1960s for this work on the discovery of Rauss sarcoma virus. And it was extremely important.

But it was still a mystery as to where this cancer associated gene came from. Where did the sarc gene originate? Was it a viral gene which the virus used in some fashion to cause cells to proliferate, perhaps for its own replication? Or did it come from some other source, some other origin? And here in the story entered two researchers, Mike Bishop and Harold Varmus. Mike Bishop is now chancellor at UCSF. Harold Varmus is president at Memorial Sloan-Kettering. They did this work at UCSF, and I got quite familiar with it because I ended up working for Harold Varmus as a Ph.D student. This work was done before I got there in about 1975. And they set out to ask this question, what is the origin of this sarc-oncogene?

Where did it come from? And what they were able to show was that this Rauss sarcoma virus derived from a virus that was related to it called avian leucosis virus.

And avian leucosis virus had only those replication genes, the genes that the virus uses to produce more virus what they were able to show was that this avian leucosis virus, when it infects cells, and here's a chicken cell, and here's the nucleus of that cell, inside that cell are normal copies of the sarc gene. They were able to show that the virus actually acquires this sarc-oncogene from the cells that it infects. Now, it doesn't do that every time.

In fact, most of the time, the virus just goes in and replicates and produces more virus.

But rarely, and really it's very rarely, the virus actually acquires a piece of the sarc gene and sticks it into its own genome, thereby creating -- -- Rauss sarcoma virus, which has the structure that I showed you above with the replication genes and the sarc oncogene. And this is done through a process called transduction, which is a form of recombination.

The main point of this observation, and the really important point of this observation was this potent oncogene is present in chicken cells.

The sarc is a cellular gene.

It's not just a viral gene. It's derived from host cells of chickens, and it's not only in chickens. It's in all vertebrates including humans. So, you all have a sarc gene in your cells, too. And this was a really momentous observation.

And it led Bishop and Varmus to win the Nobel Prize back in 1989.

So, what I've just told you is that your cells carry a potent oncogene, sarc. So, why don't you all have sarcomas?

Anybody? Remember, we are still being filmed. Anybody? Yes, somebody's got their hand up, but I don't see them. Yes? Almost right. The gene is not expressed properly, OK? The virus has co-opted this gene and placed it into its own genome. And now this gene is being regulated improperly. Maybe it's expressed too well or at the wrong time, or in the wrong cells.

And some combination of those things allows the gene to cause cancer in this setting, whereas when the gene is properly regulated, and the cells of chickens or in you, it's not cancer associated. It doesn't cause cancer in its state in the cellular genome.

So, this was extremely important, and as I said, led to the Nobel Prize for these guys. RSV -- -- is what's called an acutely transforming retrovirus, acutely transforming meaning that it can transform cells, turn them from normal to cancerous quickly. And as I said, it's a retrovirus. We now know of many acutely transforming viruses of animals: mice, rats, chickens, turkeys, other things.

And from them, we have learned the identity of about 50 or so oncogenes, all of which were derived from the host cell. So, we know from this type of experiment of many potential cancer associated genes. Importantly, there are no known acutely transforming viruses of humans.

Importantly, the vast majority of human cancers are not associated with viral agents. Occasionally, there are such cases, but they are quite rare. Most major tumor types are not caused by viral infection. But there is one big exception to this -- -- human papilloma virus.

Human papilloma virus, or HPV, and specifically high-risk types of HPV called type 16 and type 18 are associated with cervical cancer in the US, but mostly in other parts of the world. And that's important, because if one can control exposure to HPV, or eradication of HPV, one could do something about cervical cancer, and that's actually happening now. And I'll mention that again next lecture. But I want you to remember that most cancers, human cancers, are not virus associated. But the viruses have taught us a great deal about cancer genetics. OK, so if most human cancers don't arise from a viral infection, the infection with an oncogene carrying virus, then where do these mutations come from?

What are the mutations in human cancer? And here, just after these experiments were done, around 1980, comes another one of our local heroes, Bob Weinberg, who has a laboratory at the Whitehead Institute here at MIT.

He hasn't yet won the Nobel Prize, but I think he's going to, based on this work and a lot of other work probably in the next several years.

And what Weinberg's lab did back in this early 1980s.

Was to look in human cancers, so not cancers of animals, but in human cancers. And specifically, they took bladder cancers and isolated the cells, and grew them in the laboratory.

They assumed that inside of the cells were altered genes, and to become altered through the life of this individual, giving rise to this cancer. And they wanted to know what those genes were. And so, they isolated the DNA from these bladder cancer cells, and they transfected it into cells from mice. They sheared it up, and they introduced it. That's what transfected means, into the cells of mice.

And these cells that they put them into were fairly normal -- -- mouse cells, by which I mean they grew a single layer on the bottom of the dish.

There are structures look like normal cells as opposed to cancer cells. They were relatively normal cells. And what they observed was that a small percentage of these cells that had picked up some bits and pieces of the DNA derived from this human cancer began to behave abnormally -- -- and produced what were referred to as transformed cells, cells that looked more like cancer cells. So they had converted otherwise normal mouse cells to what looked like cancer cells through the introduction of one or more human genes.

And they further demonstrated that these were cancer associated cells, cancer-causing cells, by introducing them into immunocompromised mice, and observing that over time these cells could produce tumors.

So these were cancer cells indeed. So that's very interesting because it says that there are altered genes in these cancer cells that can convert normal mouse cells into cancer cells. And then to obvious question was, what are those genes? What is the gene that's responsible for this process? So, they -- -- isolated the human DNA from the transformed mouse cells.

And I won't tell you how they did that, but suffice it to say, it's possible to isolate the human DNA away from the mouse DNA in these transformed mouse cells. They cloned the human DNA into bacteria using recombinant DNA methods that we've talked to you about previously.

And they sequenced the cancer-causing gene.

It turns out it was just a single gene that was providing this property.

And it turned out that this gene was H-RAS, RAS being a gene that we've talked to you about before, and important signaling protein in cells. Moreover, they sequenced the copy of RAS that was present in the cancer cells, and compared it to the sequence in normal cells in the other cells of this individual.

And they found a single alteration. OK, so what you are looking at here now is the sequence of the H-RAS gene in normal people or in the normal cells of this cancer patient has a particular sequence.

It encodes a protein, H-RAS, which if you recall is an important signaling protein that shuttles between a GTP-bound form and a GDP bound form. In its GTP bound form, it's active and signals, and then it undergoes hydrolysis reaction. So, the GTP is converted to GDP, and becomes inactive. The mutant copy found in the cancer cells and in these transformed mouse cells has been alteration which blocks the ability of the proteins undergo GTP hydrolysis. Therefore, the protein gets locked into this active signaling state, and stimulates proliferation continuously.

And that's why it's a cancer associated gene.

OK, so here we have for the first time evidence that a normal, cellular gene gets mutated, presumably by an error or by exposure to some mutagen that is responsible at least far an aspect of the transformed state. OK, so that's very, very interesting. It also raises an important question.

If you note that the change between the normal gene and the abnormal gene is signal-base change that converts this alanine residue, sorry, this glycemic residue to a valine residue: single base change.

Such single base changes occur in your cells all the time.

It's estimated that the mutation rate inside your cells is about ten to the minus ninth per cell division. And if you calculate how many cells you have in your body at any given time based on how many cell divisions have arisen, we can estimate that there are about ten to the third to ten to the fifth -- -- rasmutin cells in all of U as you sit there now. You are all carrying 1, 00-100,000 cells that have this very mutation. So, why don't all of you have cancer? Why don't all of you have bladder cancer or some other form of cancer? Anybody?

The reason is that cancer is not a single step process. Cancer cells arise from normal cells to the acquisition of many mutations that occur over the lifetime of the individual. So a single mutation in RAS is not enough to give you a cancer. It might be enough to cause the cells to behave somewhat abnormally. But that in and of itself does not produce even hyperplasia necessarily, let alone an adenoma, an adenocarcinoma and invasive cancer.

It's necessary to acquire multiple mutations. So you may have cells that are on their way. But they are not fully there.

And hopefully they'll never get there. Now, I want to remind you that this pathway, this signaling pathway that involves RAS is something we taught you about previously. This should look familiar based on the cell signaling lectures that we had before.

RAS sits right here, this important signaling molecule that links events that take place at the cell membrane.

Sound at all familiar? Yes? Transmembrane receptors?

Growth factor receptors that bind to ligands and cause a series of events that for example convert RAS to its GTP bound form, and then initiate a kinase cascade of protein kinases, map kinase, kinase, kinase, kinase, kinase, kinase, remember the little movie? Moves into the nucleus, phosphorylates transcription factors that initiate the expression of target genes that, for example cause cells to proliferate.

And when RAS gets stuck in its on state as it does here, these signals are sent constitutively so that the cell is being told to divide all the time instead of in a regulated fashion.

Now, I point out all the different components which I've told you about in previous lectures because, in fact, it's not just RAS, this one signaling protein that gets mutated in cancers.

Many different of these signaling proteins get mutated in cancers.

It is a very common pathway that's affected in not necessarily all but a very high percentage of cancers of different types.

The RAS gene is mutated at about 30% of cancers.

30% of all cancers have mutations in one of the RAS genes including, for example, 90% of pancreatic cancer, 50% of colon cancers, 30% of lung cancers carry mutations in this gene.

But other components get mutated as well. For example, the growth factor or growth factor receptor genes get altered in cancers. They become amplified. And I'll mention what I mean by that in a second period. We observe translocations, these chromosomal alterations in different genes, including the transcription factor genes that lie at the bottom of this pathway. There can be deletions that affect the function of these proteins, make the function abnormally, and subtle mutations like the one in RAS itself. And there are other examples, subtle mutations that lock the proteins, for example, in an active signaling state.

So, DNA amplification is one such mechanism to alter the function of a cellular gene in cancer. And an example of that is in a case of a gene called HER2.

It encodes a growth factor receptor like item number two up here.

It encodes a growth factor receptor. And when it's present in single copy or two copies per cell, it produces a certain concentration of this growth factor receptor protein. And it signals at normal levels. And that gives rise to a

normal amount of proliferation. And this is particularly important in cells of the mammary gland. Mammary epithelial cells depend on this signaling pathway to be produced in the right amounts.

Unfortunately at some frequency in cancer cells, and specifically cancer cells of the breast, and sometimes ovary, an amplification event takes place so that now instead of having just one copy of this HER2 gene on the chromosome, there are many copies.

An error takes place in the replication process so that instead of just copying this gene one time, it gets copied multiple times.

So, now we have too many copies of this gene, and therefore in these cells, the concentration of this receptor is significantly higher than in normal cells such that in these cells, there might be ten times or a hundred times the amount of signaling, or ten to a hundred times the amount of proliferation. So, the dysregulation of this gene's function by making too much of the protein, it's an otherwise normal protein. There's just too much of it, causes the cells to get overstimulated and divide too often. That's bad news for cancer. The good news is we now have a therapy, and I'll put you about that next time, directed against this protein.

I'll mention another mechanism of activation, and that's translocation. I showed you slides of cancer cells that have broken chromosomes or rearranged chromosomes.

Those rearrangements often produce abnormal genes, sometimes fusions between one gene and another. Other times, rearrangements of the promoter of the gene such that the normal promoter of the gene, which is responsible for its level of expression is replaced by a different promoter, which might give too much expression. And that's true for an important oncogene called MIC. The MIC oncogene is a transcription factor. It's like number eight down there.

It's a transcription factor that binds to DNA and regulates the expression of other genes. And it's normally expressed from a, let's call it, weak promoter. You don't want to have too much of this protein produced. It needs to be produced at normal levels. So, we get a certain concentration of the protein, which gives -- -- regulated cell division. You get as much television as you need based on the appropriate signals in the environment of those cells. In certain types of leukemia, one sees a translocation.

The DNA is broken and rejoined so that the MIC oncogene doesn't have its own promoter anymore. Instead, it's next to a very strong promoter.

And now, again, the concentration of this protein is increased. And this gives rise to deregulated cell division, same idea. Change the proper regulation of this signaling network, and now the signaling network is broken. And

as I said, there are many, many such genes that are known.

We know about 50 or so oncogenes from these acutely transforming viruses. We probably know of 50 more oncogenes because of their changes in the DNA of cancer cells. And by the way, there's a lot of overlap between these two sets. Many of the genes that were found in the context of viruses were later found to be mutant in human cells having nothing to do with viruses. And RAS is one such example. MIC, actually, is another example, and a relative of this HER2 gene is another example.

OK.

OK, so the slide is meant to reinforce this notion that cell division, normal production of cells, is tightly regulated through these signaling networks, and that oncogenes function normally, their normal cellular function is to control this process.

That's what they do in normal development. That's what they do in normal individuals. But they can be subverted in cancer cells by mutation. It makes them act in a deregulated or uncontrolled fashion. There is another set of genes that act, in a sense, in opposition to the oncogenes.

These genes function to inhibit this process of cell division.

And they go by another name: tumor suppressor genes.

Tumor suppressor genes function to block the normal cell division process or to inhibit the production of cells. And they, too, are important in cancer. You can think of these genes as the on switch and the off switch of an electrical circuit.

In cancer cells, oncogenes become hyperactive.

When the lights switch gets stuck in the on state. OK, it gets taped open. So now, you're getting signaling through this circuit continuously. Tumor suppressor genes normally inhibit cell division. And so, in cancer, these genes get inactivated. It's like the light switch getting turned off.

And the process is normally regulated that way, and these get stuck off. OK, so want to tell you a little bit about tumor suppressor genes now which is the other major category of cancer associated genes that we'll cover here. Briefly, just to reiterate that point, in addition to the light switch analogy, people often use the gas pedal and breaks analogy, or the go signal and stop signal analogy. So, I'll use that as well.

In normal cells, when they receive the signals to divide, they are stimulated to go, that is, to divide to make more such cells. This happens in development when you need to make more cells. It happens in wound healing.

It happens in normal homeostasis. And then, when the process is completed, there are stop signals sent. The go signals represent oncogenes, normally regulated, stop signals tumor suppressor genes normally regulated. In cancer, oncogenes become deregulated. And pushed too strong they send constitutive signals.

And as a consequence, too many cells are produced.

And likewise, the stop signs, the stop signals are lost so that one produces even more cells. OK, so hopefully these are useful analogies to think about these two opposing sets of oncogenes and tumor suppressor genes. OK, so to get into tumor suppressor genes, sorry, this just makes the point, oncogene mutations, tumor suppressor gene mutations. To get into tumor suppressor genes, the second class, an important class of human cancer genes, let me tell you a little bit about this disease which is called retinoblastoma. Retinoblastoma is a childhood tumor of the eye, of the retina. It's not very common. Only about one in 40,000 children develop this tumor. But it's very important what it's taught us about cancer genes. And you can see in the normal eye a normal looking retina, and in the cancer containing eye, these blobs of tumors. We now know the gene that's responsible for this disease, the gene that's mutated in the formation of this disease.

And that gene is the gene called RB, named after retinoblastoma.

And this gene functions as an important regulator of cell cycle progression. If you remember cell cycle progression, mitosis, S phase mitosis, the G1 phase, the G2 phase, again, dim memories from earlier in the class. The RB protein, which goes by the name of PRB, this is the protein encoded by this tumor suppressor gene, RB, acts to inhibit cell cycle progression. That's its function. It blocks cell cycle progression literally like the brakes on a car. Now, you might ask, if it's there and functioning, then how do you ever get cell cycle progression? And the answer is that this active inhibitor can be inactivated through phosphorylation. When this protein gets phosphorylated, it becomes inactive.

And now, cell cycle progression can occur.

It becomes inactive when the cell receives growth stimulatory signals like through that signaling network that I mentioned previously.

These signals, then, act on cycline CDK complexes, cell cycle associated kinases that you were taught about in the cell cycle lecture, and these go about inactivating the RB protein, allowing the cells to cycle.

In addition, when the cells shouldn't be cycling in the presence of growth inhibitory signals, inhibitory signals are sent to the cycline dependent kinase proteins, and they don't function, thereby blocking the inactivation of RB. RB stays in its active state and blocks cell cycle progression. OK, so that's how this really important cell cycle

regulator functions more or less.

And it's a very important human tumor suppressor gene, which is itself mutated and probably 30 or 40% of human cancers.

And the pathway that regulates this gene, this pathway, is mutated in probably 95% of human cancers very, very commonly affected.

OK, so given what I've told you so far, would you expect the mutations that we find in the RB gene in human cancer to be activating mutations or inactivating mutations? Inactivating. It's the brakes.

Cancer cells want to get rid of the brakes.

And so we find inactivating mutations in the RB gene in human cancers like deletions or nonsense mutations, that is, stop codon mutations.

OK, that's the kind of mutations we find in this gene.

For the oncogenes, which we focused on at the beginning, are the mutations in oncogenes dominant or recessive?

Are the mutations in the oncogenes dominant or recessive?

The oncogene mutations are dominant. When you acquire mutation in an oncogene, whether it's a subtle mutation or an amplification, it doesn't matter what's happening to the other allele.

This thing has new function, gain of function. It will transform or start the process of transformation, regardless of what is happening to the other allele.

And that's evident, by the way, in this transfection experiment. This would only work if it were a dominant mutation.

So, oncogene mutations are dominant mutations. Are tumor suppressor gene mutations dominant or recessive? These are recessive mutations.

Here, it matters, the state of the other allele of the RB gene. As long as you have one functional copy of the brakes, you can do this. You can control cell cycle regulation.

In order to make this dysregulated, you need to get rid of both copies.

You need to get rid of the brakes entirely. So, these mutations are recessive. And this class of mutations are recessive. Tumor suppressor gene mutations are recessive.

So this raises an interesting question. Tumor suppressor gene mutations are recessive. I've told you that tumor suppressor gene mutations occur commonly in human cancer, not just this RB gene but others too, and yet you are all diploid.

You all carry two copies of the tumor suppressor genes.

So, how do we ever find mutations in these genes in human cancer?

Is it enough to mutate just one copy? No, because they are recessive mutations. So, to find mutations in tumor suppressor genes in human cancer, it's not enough to mutate just one copy. Both copies need to be mutated.

Recessive mutations require there be two mutations. And this is what typically happens in the development of a tumor with a mutation in the RB gene, or any other tumor suppressor gene. Again, there are probably 20 to 50 different tumor suppressor genes. Initially, a cell, which has a normal copy of RB on both copies of chromosome 13, that's where this gene sits, and here we are looking at a linked polymorphic marker, big A little a, if you remember polymorphic markers, big A little A.

First, one copy of RB gets mutated. In this cell, there is still a normal copy of RB. And therefore, this cell that has acquired this mutation is normal.

It's functionally normal. However, it now has just one copy of the RB gene. And so, it's predisposed to becoming a cancer cell if that normal copy gets lost.

And what this shows you are various mechanisms by which the normal copy of the RB gene can be lost.

The other copy can acquire its own mutation. Or, the chromosome that carries the normal copy of the RB gene can get lost in the developing cancer cell. Or, it can be a recombination of that, that replaces the normal copy of the gene with the abnormal copy of the gene. And all three of these things happen in the development of cancer cells. And depending on which mechanism is used -- -- one can observe something referred to as loss -- -- of heterozygosity, loss of heterozygosity within the cancer cell. You will notice that this cancer cell, sorry, this normal cell, is heterozygous for this A allele, big A little A. And you will notice that here and here, the cell has become, the cell carries only one version of that A allele, big A. So, there was heterozygosity.

And in the cancer cell, there's now loss of heterozygosity.

Loss of heterozygosity is a hallmark for tumor suppressor genes, the involvement of tumor suppressor genes in the development of cancer.

OK, now I told you several times that proliferation is important and cell death is important, too.

Cell death control in cancer: I don't have a lot of time to tell you more about this in this class, but I want to just emphasize that the pathways that lead from cell death signals, the signals that tell cells to commit suicide to ultimately undergo this process called apoptosis are also dysregulated in cancer.

For example, there's a gene called BCL2, which becomes mutated in certain types of cancer.

BCL2 normally blocks this process of cell death.

And in this type of cancer, it becomes hyperactive. So there's not as much cell death. BCL2 is an oncogene.

Another gene, very important gene in human cancer is P53.

It stimulates cell death. P53 gets lost in a high percentage of cancers. It's a tumor suppressor gene, and therefore gets inactivated. BCL2 is an oncogene.

It gets hyperactivated in cancers. I'll talk more about this pathway next time, but just to put in your minds, P53 is a very important signaling protein responsive to many signals upstream, giving rise to signals that lead to death as well as arrest.

Hang on, please. Hang on. Two minutes left.

And this slide is just a summary of what we've been talking about so far.

Control of proliferation, control of cell death, the normal balance, perturbations in these pathways, oncogene mutations that stimulate proliferation, tumor suppressor gene mutations that stimulate proliferation. Alterations in apoptosis, oncogene mutations that block apoptosis, tumor suppressor gene mutations that block apoptosis. All of these are important in cancer. Next time I'm going to tell you that some cancers are caused by inherited mutations. And this is a pedigree of such an example, and lastly to remind you, mutations affect proliferation in cell death. But they also affect many other things: invasion, angiogenesis, as well as metastasis.

And I'll stop there.