Eukaryotic Gene Expression: Basics and Benefits

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Module No. # 05

Lecture No. # 19

Regulation of Gene Expression by Growth Factors

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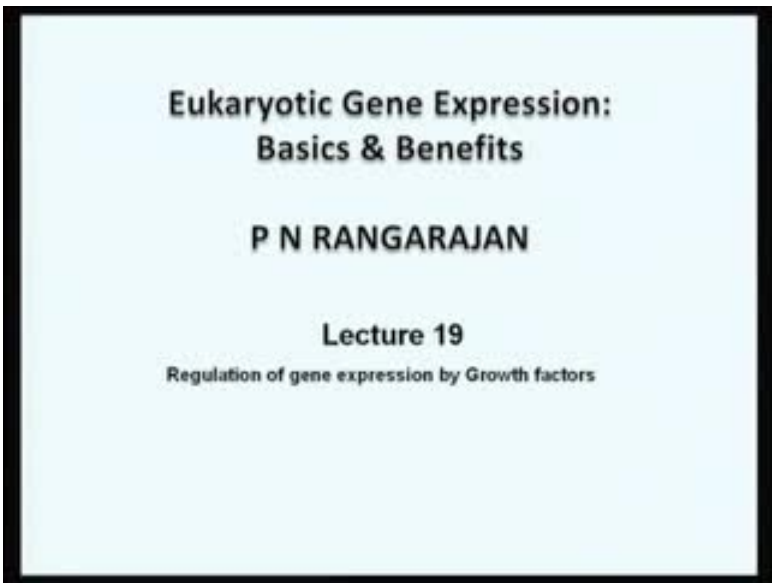

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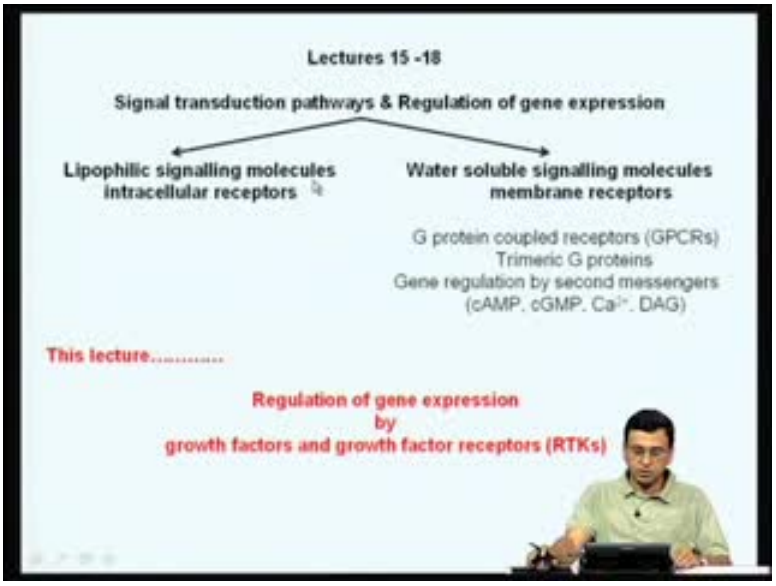

Welcome to this nineteenth lecture in this course on eukaryotic gene expression, basics and benefit's. Today, we are going to discuss about regulation of gene expression by growth factors. Just we recapitulate what we have been doing in the last few classes. Since last four classes, we are primarily discussing about signal transduction pathways and regulation of gene expression that focuses primarily is to see how gene expression inside the nucleus is modulated by molecules which either enter the cell through the cell membrane or molecules which stay at the cell membrane. And what we have been discussing in a last few classes is that molecules such as lipophilic signaling molecules. They can simply diffuse through the cell membrane and interact with specific cells intracellular receptors and these receptors can then, go on and bind to specific sequence on the promoters of organ genes and other activate or repression transcription.

We still have not discussed in detail this particular pathway, but what we are actually discussing the last few classes is about another set of signaling molecules which are rather water soluble and therefore, they cannot pass through the lipid bilayer therefore, they stay at the cell membrane and interacts with specific membrane receptors and when these molecules interact this membrane receptors, that results in what is called as a signal transaction events ultimately living to the activation of specific protein kinases and these kinases then go inside the nucleus phosphorylates transcription factors which in turn either activate or repress transcription of specific genes, related to specific philological responses.

Now, signaling by membrane receptors is a huge area of research, a number of signaling molecules activate gene expression through this pathway. We actually began discussing one set of molecules which interact with specific receptors called as the G protein coupled receptors of GPCRs, which are seven trans membrane proteins and what we discussed is one molecule like epinephrine or glucagon, when they interact with such cell membrane GPCRs, it then resist in the activation of Trimeric G proteins which consists of alpha, beta and gamma sub units and then the alpha subunit dissociates from beta gamma sub unit. The alpha sub unit then goes and activates molecule such as protein kinase, sorry phospholipase C or it can activate protein kinase A or it can activate protein kinase C and depending upon what kind of G alpha subunits are interacting with these, we have specific second messengers being activated.

For example, when G alpha subunit interacts with protein kinase A, it results in the synthesis of cyclic AMP. When the G alpha subunit interacts with phospholipase C, it results in the production of calcium and diesel glycerol, which go on then all these acts as second messengers. And we also discussed about guanylyl cyclases which unlike the other once is an intrinsic membrane receptor having a guanylyl cyclases activity and that also gets activated by specific G protein couple receptors and that results in synthesis of cyclic GMP, again access a very important signaling molecules. So, we basically covered one particular group of signaling molecules which activate or which activate gene expression programs through GPCRs or G protein coupled receptors.

What will do in this lecture is to discuss another class of signaling molecules which activate gene expression programs through receptors known as receptor tyrosine kinases or RTKs. These are the receptors which have intrinsic tyrosine kinase activity therefore, when this signaling molecules bind to the receptors the tyrosine kinase activity of the receptor is activated and let us see how activation of this tyrosine kinase activity can pass through signals ultimately into the cell leading to the activation or repression of transcription. Now, this kind of signaling pathway that we are going to discuss today is primarily mediatory by molecules known as growth factors. So, let us now try to understand what are these growth factors, what kind of growth factors are influence gene expression programs through this particular pathway involved in the receptor tyrosine kinases.

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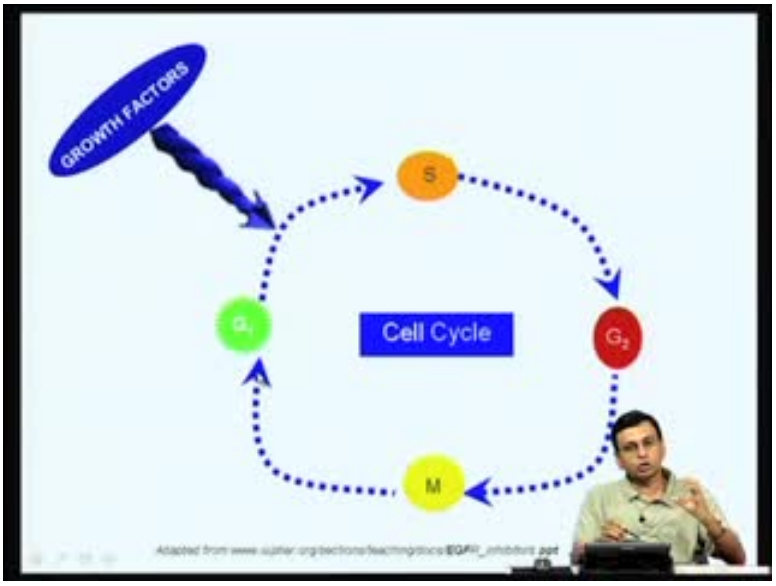

Now, growth factors play a very important role in promoting cell proliferation and we all know cells actually cycle and this is what as cell cycle and when cell cycle, they usually enter from G gap one phase, when cells actually grow increase in size and make sure all the raw materials for the cell replication of DNA as well as the other processes are present and then from the G 1 phase, cells enter the S phase then, go to the G 2 phase then, enter the mitoses. This is what is called as cell cycles. So, growth factors primarily activate the cell cycle program and make cells come it from G 1 to and enter the cell cycle and this is what cause induce the cell division leading to cell proliferation. So, what will now discuss in the next few minutes is to understand, how this growth factors induce cell cycle, make cells to come it from G 1 to S and how the cell cycle propagation is initiated by growth factors, leading to cell proliferation and cell growth.

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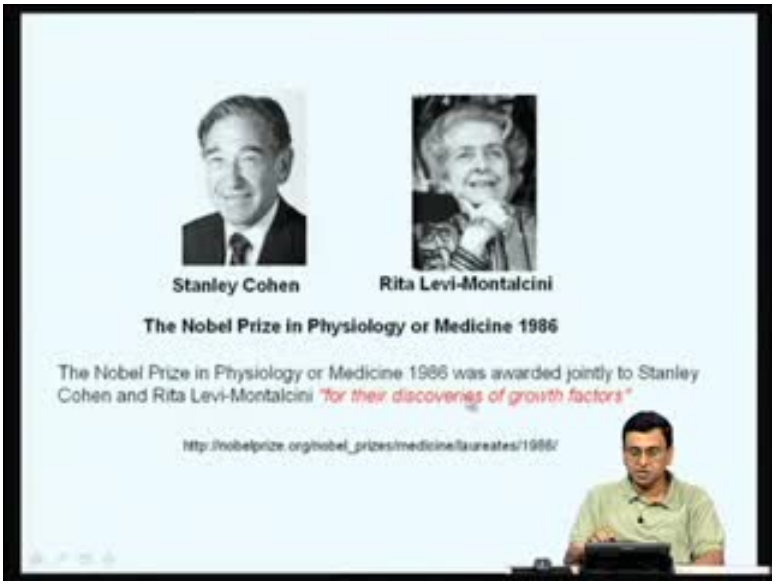

Now, let us go to a brief history of how growth factors were actually discovered because very important for us to understand appreciate how the field has grown where the last few years, ever since the discovery of growth factors by two very important pioneers in this field, Stanley Cohen and Rita Levi-Montalcini. These are the two people to whom the credit for discovering growth factors goes and to acknowledge their contribution to the field of science to the beautiful biology in the medicine, noble prize was given to them in the year 1986. So, as you can see the citation the noble of prize website says, the noble prize for physiology and medicine 1986 was awarded jointly to Stanley Cohen and Rita Levi-Montalcini for their discoveries of growth factors. So, growth factors are very important and the discovery of growth factors has been acknowledged by awarding noble prize to these two people. So, let us now see what exactly this people did and how exactly growth factors were discovered.

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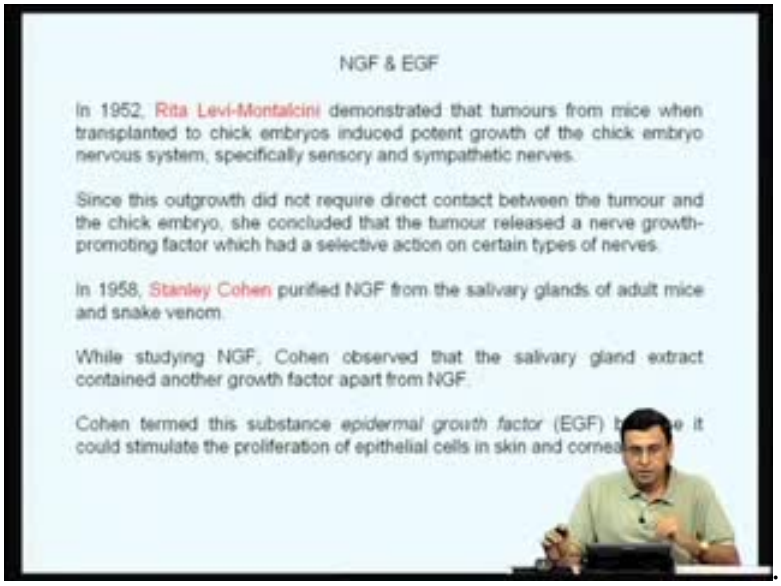

Now, sometime in 1952 Rita demonstrated that tumors from mice when transplanted to chick embryos induced potent growth of the chick embryo nervous system, specifically sensory and sympathetic nerves. So, when she took the tumors from mice and the implantation of chick embryos, it promoted the growth of nervous tissue in the chick embryo. But an important observation that she made is that this outgrowth did not require direct contact between the tumor and the chick embryo. Clearly indicating that the tumor actually, released a specific nerve growth promoting factor which induced growth of nerves in the chick embryo.

So, some kind of a diffusive molecule is coming out of this tumor cells that is what is acting on this chick embryo tissue and then inducing the proliferation or inducing the formation of nerve tissue. So, this work was going on. Around 1958, Stanley Cohen actually purified this factor, she called it as nerve growth factor because it was such a promoting the growth of nerves. So, 1958 Cohen actually purified this nerve growth factor from the salivary glands of adult mice and snake venom. And you also note another important observation that while actually studying this nerve growth factor from the salivary purified from the salivary glands, he observed that salivary gland extract contains another important growth factor and he called, which is different from that of the nerve growth factor and he called it as epidermal growth factor or EGF because it basically stimulate the proliferation of epithelial cells in the skin and cornea.

So, this is how this marked the beginning of discovery of growth factor research. So, Cohen and Rita Levi-Montalcini are the two people who discovered two important growth factors namely, the nerve growth factor and epidermal growth factor. And this initiated the growth factor research and today, we have whole battery of growth factors and this growth factors receptors has now grown into a billion dollar industry because these growth factor signaling plays a very important role in areas like cancer inflammation and so on and so forth. So, number of pharmaceutical companies are interested in developing molecules which either activate this growth factor signaling pathway or inhibit this growth factor signaling pathway. So, that is specific gene expression programs can be either activate or repressed and it has very important biomedical applications.

So, it is very important for us to understand how these growth factors, when they interact with this growth factor receptors ultimately, initiate a signal transduction pathway leading to activation of specific gene expression program because it not only has implications in basic research, but also has a lot of importance in biotechnology. Now, the next question, once Cohen and Rita Montalcini discovered these two growth factors, the next question is how do these growth factors act? How are they able to induce the growth of nerves? How are they going to induce phosphorylation epithelial tissues?

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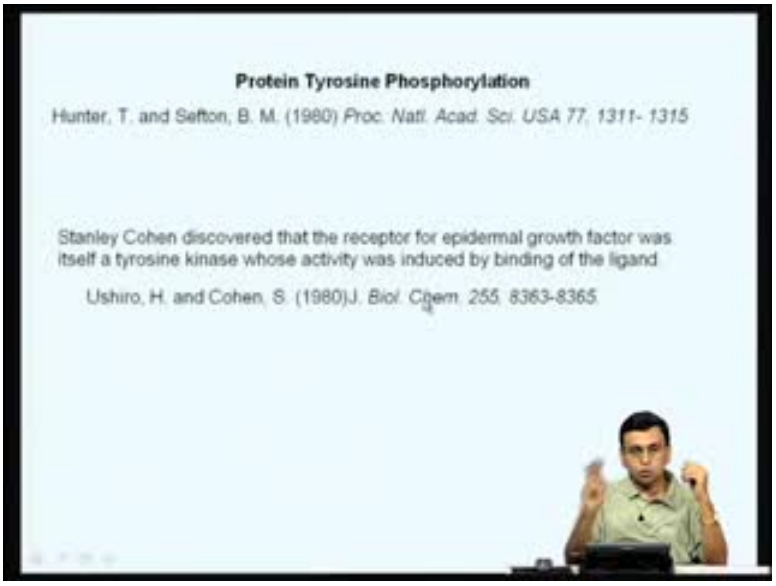

It turned out two important discoveries paved way for understanding this mechanism of action of growth factors. One is Tony Hunter and Bartholomew Sefton working in Salk Institute in San Diego discovered that proteins can be phosphorylated and tyrosine residues. Since then, phosphorylation of proteins on serine and threonine residues are known, but for the first time Tony Hunter actually showed, there are protein kinases which can phosphorylate tyrosine residues and this mark the another very important area of research called as discovery of receptor tyrosine kinases.

The discovery of tyrosine kinases another important landmark in the area of biology and medicine because it again has very important implications, in both biology as well as medicine. And once around the same time when these people discovered the tyrosine phosphorylation and tyrosine kinases, Cohen actually observed that the receptor for epidermal growth factor, in fact, what is itself is a tyrosine kinase and it activity can be induced by the binding of the ligand. This is the paper published by Cohen's group demonstrating that the receptor for the epidermal growth factor of the EGF receptor or EGFR is a tyrosine kinase and when the growth factor binds the growth factor receptor the tyrosine kinase activity of receptor is activated. So, these are the two important landmarks in the area of growth factor research and this actually mark, this opened up a new entirely new area of research to understand how receptor tyrosine kinases play very important role a number of cellular processes.

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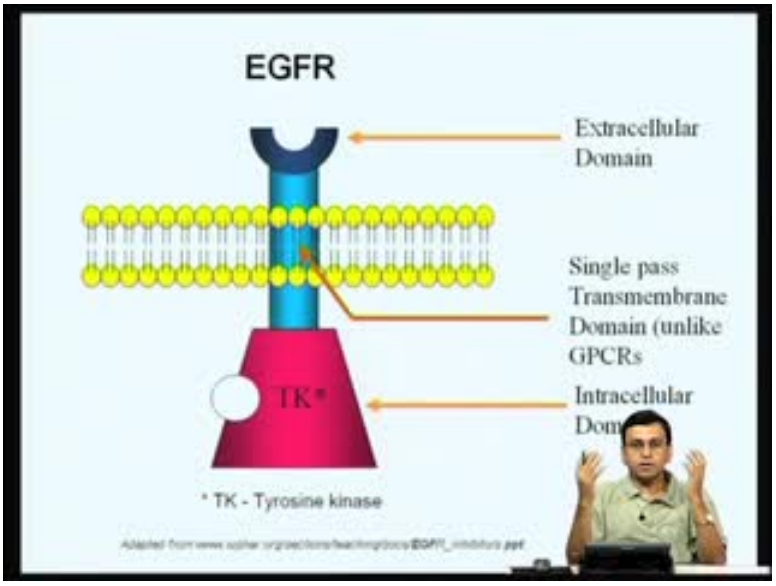

So, let us now try to understand how exactly the EGF signaling takes place, what happens when epidermal growth factor binds to the epidermal growth factor receptor. So, now shown in this cartoon, it is actually taken from a very nice website. So, I have just take this slide from this website which very nicely shows for example, the epidermal growth factors receptors basically contains three important regions. One is the extracellular domain to which the epidermal growth factor is comes and binds and it also contains a trans membrane domain. I would like to spend a couple of seconds here, just to tell you that this trans membrane domain of the epidermal growth factor receptor is different from the G protein couple receptors which you have studied in the last three classes.

Now, the GPCRs of a recall actually contains a seven trans membrane domain. That means, this trans membrane domain traverses the cell membrane seven times. So, these are called as the serpentine receptors of the seven trans membrane receptors. Unlike that, the receptor tyrosine kinases contain a trans membrane domain which traverse the membrane only once. So, it is called a single pass trans membrane domain. It is a very important distinguish between the GPCRs or G protein coupled receptor on the receptor tyrosine kinases. They contains serpentine or 7 trans membrane domain. This contains only one trans membrane domain. The third important domain that these receptors contain is as called as the intracellular domain and this intracellular domain which actually faces the cytoplasm is actually contains the tyrosine kinase activity.

So, the entire growth factor signaling on two things. What happens in the growth factor binds the growth factor receptor, how the tyrosine kinase activity of this receptor is activated and once the tyrosine kinase activities activated what happens next? How this tyrosine kinase now interacts with other proteins and how this results in the a phosphorylation or activation of other kinases, tyrosine kinases namely, the map kinases and which then, go on then, phosphorylates specific transcription factor leading to activation or repression of specific target genes?

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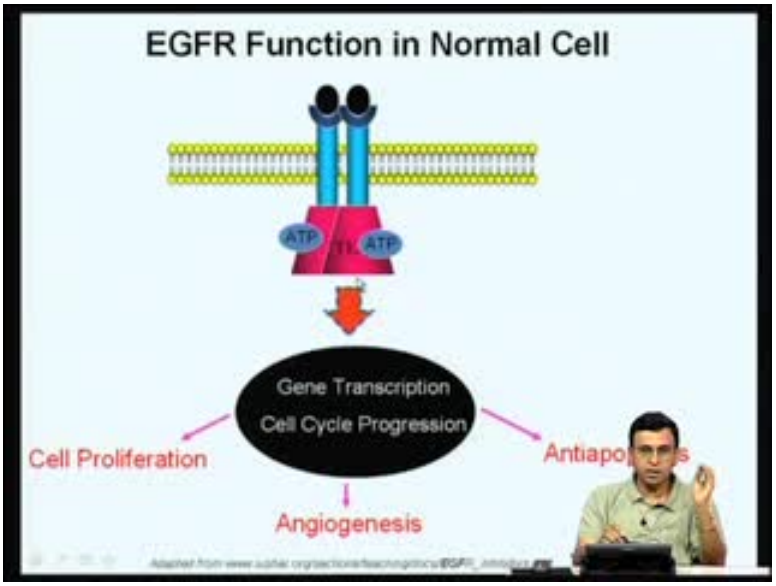

So, let us now try to understand how does the EGF receptor function in a normal cell. So, this is a carton that tells you in a very nice manner again, taken from a very nice website. Here is a cartoon that actually depicts, when the epidermal growth factor binds to the epidermal growth factor receptor, it induces dimerization of the receptor. I just go back and then click once again. This is the epidermal growth factor and you can see now the epidermal growth factor binds to epidermal growth factor receptor, it now induces the dimerization of the receptor and once the receptor dimerise form, it can now bind ATP and using the energy level ATP hydrolysis.

Now, this tyrosine kinases trans phosphorylated each other. That means, one monomer phosphorylates the tyrosine residue other monomer and this cause a trans phosphorylation. So, each monomer phosphorylates tyrosines on each other and thus the tyrosine kinase gets activated and then very important events takes place. I have shown

here as a blocked red arrow which we will discuss in detail and this activation of the tyrosine kinase activity leads to a series of events including the activation what are called as the map kinase cascade. Ultimately culminating in the regulation of gene expression inside the nucleus and depending upon what kind of genes are getting activated or repressed, you can get cell proliferation, it can get angiogenesis, you can get anti apoptosis. Now all these events have very important implications in cancer cell proliferation and finding a cure for cancer. So, epidermal growth factor as well as other growth factors are very important targets for people working in the area of cancer, inflammation, other areas of research because activation of this particular signaling pathway ultimately results in proliferation of cells leading to growth and cell proliferation.

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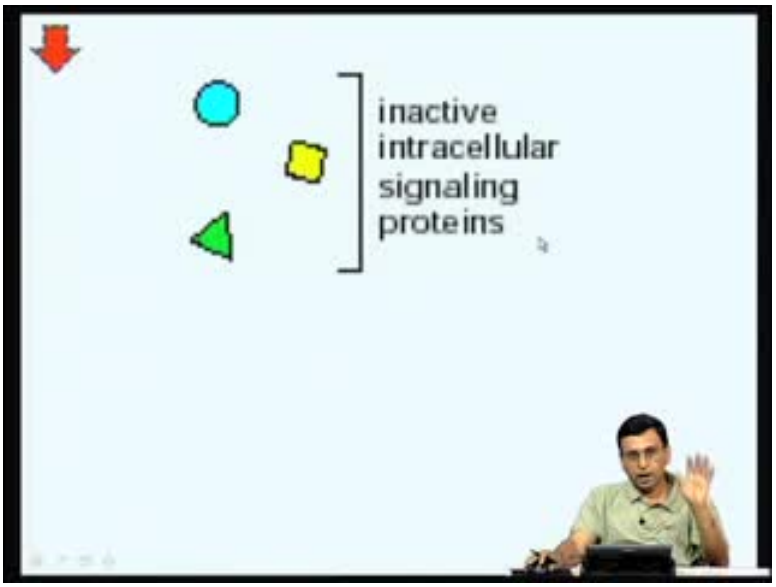

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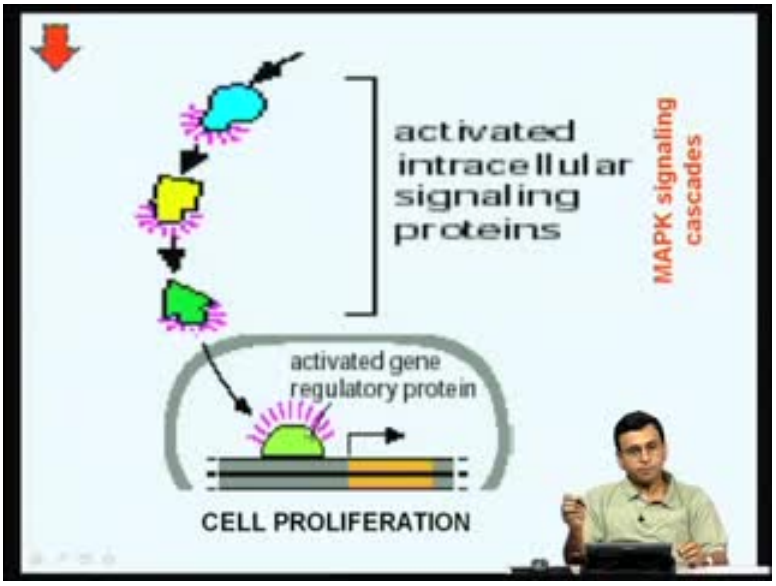

So, let us now try to understand this red arrow, what exactly happens once the growth factor bound to its receptor the receptor got activated, how does it influence events in the nucleus? Now, what basically happens is that a number of inactive intracellular signaling proteins which are actually in the inactive state and once the tyrosine kinase is activated, they all get activated and each one of them activates the membrane, the subsequent lineage of this pathway and this series of activation of series of molecules, basically is now referred to as the map kinase signaling pathway, map kinase refers to mitogen activated protein kinases.

So, these growth factors activation as mitogens because they induce mitoses and makes cells proliferate and therefore, this particular kinases which are activated the mitogens is referred to as the mitogen activated protein kinase because these protein kinase are activated by mitogens binding at the cell surface receptors. So, when the growth factor binds to the growth factor receptor, the receptor undergoes dimerization and these dimerization activates the tyrosine kinase induce a confirmation change receptor leading to the activation of the tyrosine kinase activity of receptors and once the tyrosine transducer is phosphorylated, it activates the map kinase signaling cascade and this activation of this map kinase signaling cascade, ultimately results in the phosphorylation of specific transcription factors, leading to activation of cell proliferation programs. So, this is a very briefly a nut shell by which the growth factors influence gene expression programs.

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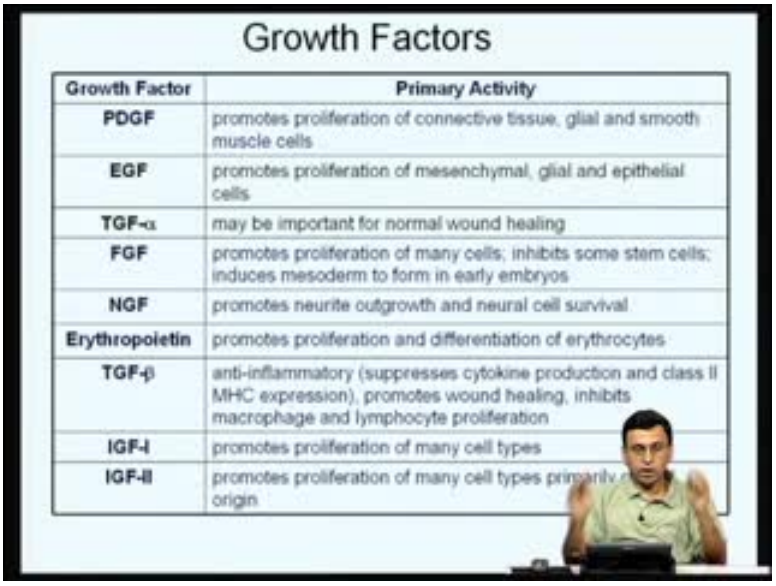

Now, ever since the discovery of epidermal growth factor as well as nerve growth factor a number of such growth factors have been discovered and they all have more or less similar scheme which have described so far. That means, they all bind to a receptor which is a tyrosine kinase and activity of tyrosine kinase is actually activated, when the hormone binds on the growth factor binds to the receptor, it promotes receptor dimerization leading to the activation of tyrosine kinase activity. Some of these growth factors are being listed here, but this is not an exhaustively there are many others. I have only listed few here. For example, you have a growth factor called platelet derived growth factor or PDGF which promotes proliferation of connective tissue glial and smooth muscle cells.

Epidermal growth factor we already discussed proliferation of epithelial cells. We have what is called as a transforming growth factor alpha, which is plays a very important role wound healing. We have fiber glass growth factor promotes proliferation of many cells in inhibit the some stem cells and induces mesoderm to form in the early embryos, plays a very important role during embryonic development. nerve growth factor again is one of the original growth factor discovered along with a epidermal growth factor promotes the outgrowth of neuritis and neural cell survival.

Erythropoietin is another very important hormone promotes proliferation differentiation of erythrocytes. the transforming growth factor beta, plays again very important role in

number of cellular passes including anti-inflammation, it suppresses cytokine production class 2 MHC expression, promotes wound healing, inhibits macrophage and lymphocyte proliferation and you have like insulin Growth Factor 1, insulin Growth Factor 2 again has very important regulation, very important physiological responses. So, ever since the discovery of epidermal growth factor and nerve growth factor a number of such growth factors have been discovered and some of this has been discovered. So, let us now spend a few minutes try to understand, how these growth factors activates gene expression program and we will use epidermal growth factor as a model or to understand how these growth factors signaling actually takes place.

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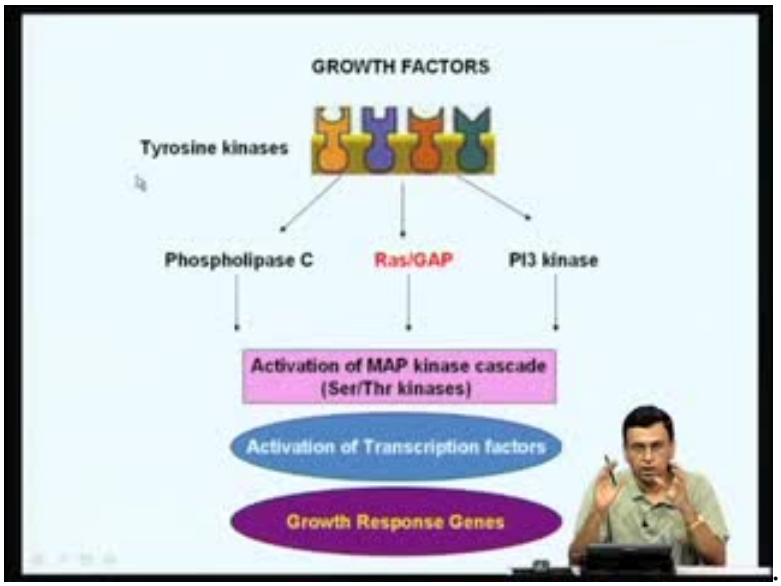

So, in general many of these growth factors not all, but many of these growth factors they have their own distinct receptors. So, each growth factor binds to its cognate receptor and one thing that is common for many of the growth factors that we are going to discuss today, they all contain intrinsic receptor tyrosine kinase activity. So, there are all tyrosine kinases. The tyrosine kinase activity is activated when these growth factor binds to it cognate receptor. Now, once the tyrosine kinase activity of the receptors activated, what kind of genes are activated or what kind of signaling pathway is activated depends on what are the effector molecule are activated by the tyrosine kinase.

In some cases the activation of the tyrosine kinase activity results in the activation of phospholipase C. In such case, different type of signaling cascade is activated, if this

growth factor receptors activated Ras protein which is a monomeric G protein then, a different kind of a signaling cascade is activated if you have P I 3 kinase has the effecter molecule and that is activated a different kind of signaling cascade is activated living to activation or repression of different kind of target genes.

So, in all these cases basically, it involves the activation of what is known as the mitogen activate protein kinases. And these mitogen activate protein kinase are nothing but serine threonine kinases, we have discussed some of this things in the GPCR signaling in the previous classes and these map kinases ultimately phosphorylates specific transcription factors leading to the activation and this transcription factors then activate many genes involved in growth such as the EGR 1, etc leading to cell proliferation and growth. So, this is the general scheme by which many of these growth factors promotes cell proliferation and growth.

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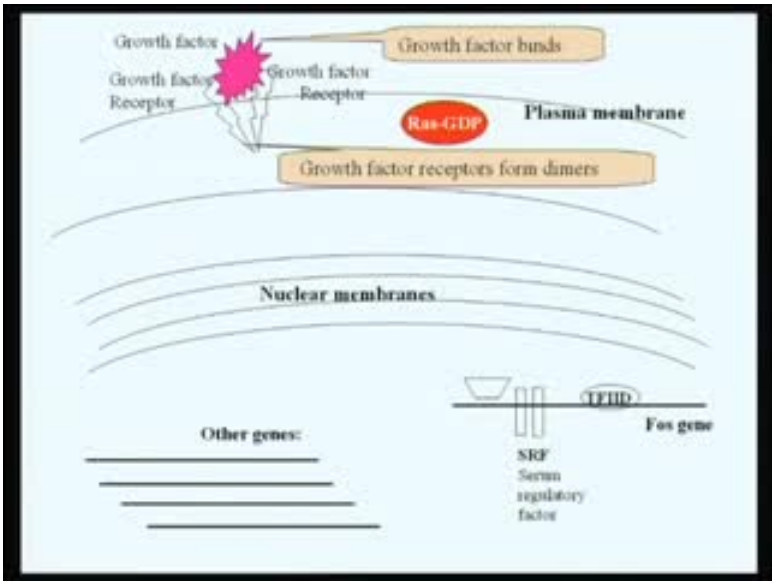

So, what I am going to next few is going to run series of cartoon, just to give you an idea what are the important biochemical event that actually takes place, so that you can easily remember the various important molecular event that takes place leading to the activation of growth factors signaling pathway ultimately, culminating in the activation or repression of genes. So, what will do in the next few slides to go through a series of schemes to understand, how exactly this signal transduction pathway is activated. So, what the purpose of this presentation is once the growth factor binds to the growth factor

receptor how does specific genes inside the nucleus are activated, what are the events between the growth factor bind to the growth factor receptor and activation of genes involved in cell proliferation. This is what we are going to discuss.

So, one thing, the first thing that happens on the growth factor binds with the growth factor receptor is the two monomers which were far apart, now they come together and they undergo dimerization. So, one when the growth factor binds the immediate consequence of the growth factor bind to the growth factor receptor is the dimerization of the growth factors and you can see here the important effector molecule in the signaling transduction pathway is the Ras GDP.

Now, when we discussing about the GPRs signaling pathway, we had discussed primarily about trimeric G proteins which contains three subunits namely, the alpha, beta and gamma and we showed the activation of the trimeric GDPs actually depends on the dissociation of the beta gamma subunit and the alpha subunit which is not active, which then, goes on interacts the number of effector molecules like adenylate cyclase or it can be phospholipase C and so on and so forth. Here what we are discussing is about a different kind of a G protein which has only one subunit and this is known as the Ras. So, Ras is a monomeric GDPs and in many ways, it has high degree of homology to the G alpha subunit of the trimeric G protein which you have studied in the previous classes. So, the purpose of the next few minutes is to understand how this Ras which is actually GDPs, converts GTP to GDP and how this Ras is activated by this growth factor bind to the growth factor receptor, what are the important events that takes place between the activation of Ras GTP and the growth factor binding.

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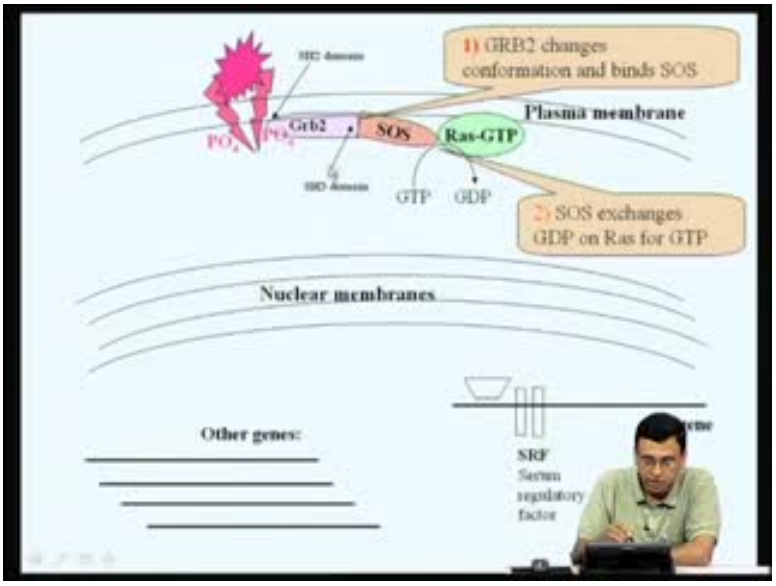

Now, once the growth factor binds to the growth factor receptor and receptors undergo dimerization the immediate thing, that happens is the trans phosphorylation. So, tyrosine residues on this particular monomer is phosphorylated by this kinase, of this kinase monomer and the tyrosine residues on this particular monomer is phosphorylated by this particular kinase. So, trans phosphorylation of tyrosine residue occurs on the monomer and this results in the activation of the receptor. Once the tyrosine residues in the cytosolic domain of this growth factors is phosphorylated, the next immediate event happens is these tyrosine phosphorylated growth factor receptors now interacts with a protein called Grb 2.

Now, Grb 2 interacts with this tyrosine phosphorylated growth factor receptor to a very specific domain known as the SH 2 domain. SH stands for Sarc Homology domain because this domain was originally identified name protein called Sarc or S r c which is protein. So, once the tyrosine residue of a growth factor is phosphorylated, this phosphorylated tyrosine since now interact with a protein called Grb 2 through this S H 2 domain. This very important for it remember because this particular interaction of this Grb 2 with phosphorylated tyrosine kinases happens through this S H 2 domains in a number of growth factors signaling pathways.

So, interaction of this Grb 2 with tyrosine residues of receptor requires these SH 2 domains. Now, what happens next? Once the Grb interacts with the, through the SH 2

domain with the phosphate tyrosine, it now interacts with another protein called SOS through another domain, now known as the SH 3 domain or the Sarc homology 3 domain. So, the SH 2 domain facilities the interaction of the Grb 2 with the receptor tyrosine kinase whereas, the SH 3 domain facilitates the interaction of the Grb 2 with SOS. SOS is another protein known as the Son of Sevenless.

Now, we will not go to the details of this because we are not really discussing the signal transduction lectures, but our primary aim is to understand how gene expression programs are activated through the signaling pathways. Now, Grb 2 once the Grb 2 interacts with the receptor tyrosine kinase through the SH domain, it under goes a conformational change and now because of this conformational change, it can now interact with SOS through this SH 3 domain. Now SOS is a very important protein and is a GDP exchange protein. That is why now is capable of exchanging G D P on Ras for GTP. So, the Ras which is in a GDP bound form, now the GDP is removed and it now is made to bind GTP. So, the regulation of Ras activity is primarily carried out by SOS and let us now understand how exactly this takes place.

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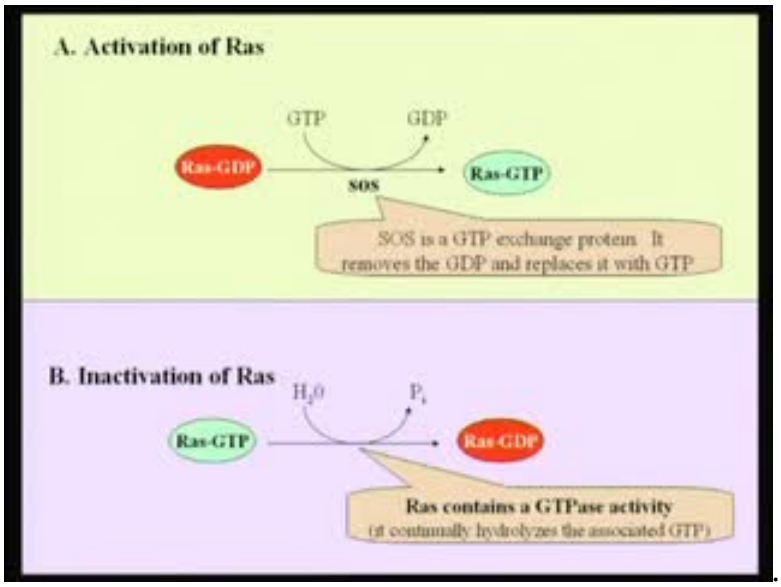

You can see Ras molecule which is basically GTPs, when it is bound to GDP is inactive. That is why I have shown it in the red. So, the inactive form of Ras when it interact with SOS, the SOS now removes the GDP and now makes the Ras bind to GTP. Now being a G T Ps, so, Ras the SOS is actually called as GTP exchange protein, it removes the GDP

and replaces it with GTP in Ras molecule. So, now we have Ras which is in the GTP bound form and now in the GTP bound form it is considered active. So, I depict in the green here and once is being a GTPs, it immediately hydrolysis the GTP and becomes again a Ras GTP. So, as long as the growth factor remains bound to the growth factor receptor, there is a continuous activation of this trans phosphorylation tyrosine residue occur. The Grb 2 interacts with the phosphorylated receptor then, SOS interacts under source now continuously exchange a GDP to GTP and this signaling continues as long as the growth factor remains bound to the receptor.

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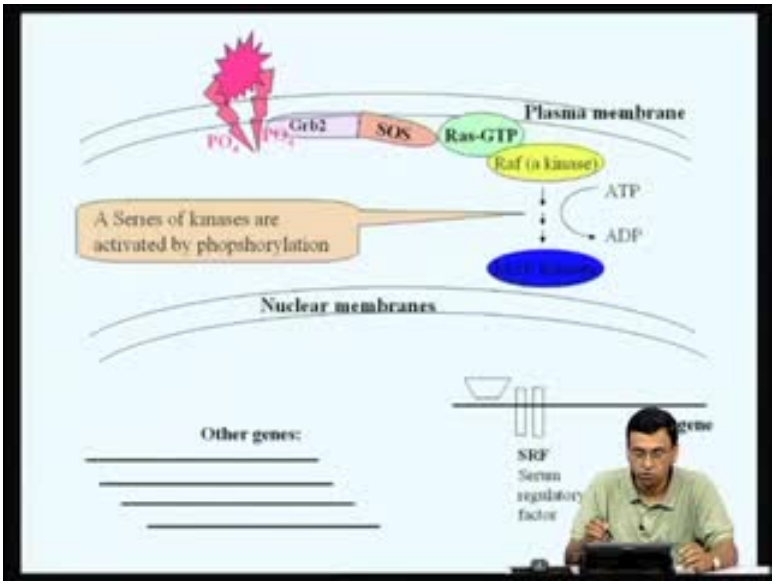

Now, once the Ras is activated, now the immediate consequence is that Ras now activates a protein called as a Raf, which is actually a kinase and this Raf now, activates a series of kinases and this is known as the map kinase cascade. So, will spend some time to understand how this map kinase cascade is activated by Ras. So, hope this particular important events are clear. I will go through this once again. Once a growth factor binds to a growth factor receptor the two monomers of the growth factor dimerise and once they dimerise the tyrosine kinase activity is activated and each monomer trans phosphorylates the tyrosines of the other and once the tyrosine growth factors are phosphorylated, it interacts the protein called Grb 2 through specific domains known as the SH 2 domains and now the Grb 2 interacts another protein called SOS. It stands for Son Of Sevenless, through another important domain called SH 3 domain. And SOS is known as the GDP exchange protein. It exchanges a GDP for GTP in the case of Ras and

once Ras is in the GTP bound form, it hydrolyses and GTP hydrolyses, now results in the activation of Raf which is a kinase and this now Raf, now goes on activates other series of kinases are in the activation of the map kinase cascade.

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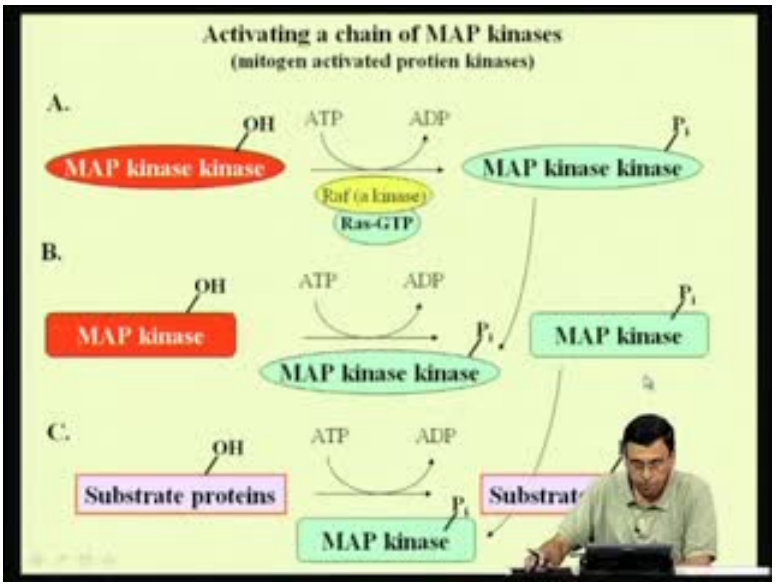

So, we will now come to the second phase of this activation pathway. We have now finished one pass. So, growth factor bind to the growth factor receptor activation of Ras. Now Ras results in the activation of the map kinase pathway. Now, how does the map kinase pathway gets activated. The first one is known as the map kinase kinase which being inactive form is activated by Raf and Ras GTP. Now becomes active once this phosphorylated it now becomes active. Now, phosphorylated map kinase kinase now activates the next in the series the map kinase whose hydroxyl group of the serine now gets phosphorylated. So, we have got an active map kinase and this map kinase, now goes inside a nucleus and phosphorylates a number of substrate proteins which could to transcription factors so on and so forth.

So, activation of this map kinase pathway is a very important event in the receptor tyrosine kinase signaling or the growth factor signaling. So, once through series of phosphorylation's different map kinases are activated and finally, the last map kinase now goes and activates phosphorylates specific transcription factors activating and this transcription factors now, go and bind to specific target genes resulting the cell proliferation program. So, we are now discussed of the three important events in the

growth factor signaling. We have discussed two events. Once is the growth factor binding to the growth factor receptor leading to the activation of the Ras protein and once the Ras protein is activated, it now results in the activation of a map kinase. And let see what does this map kinase do?

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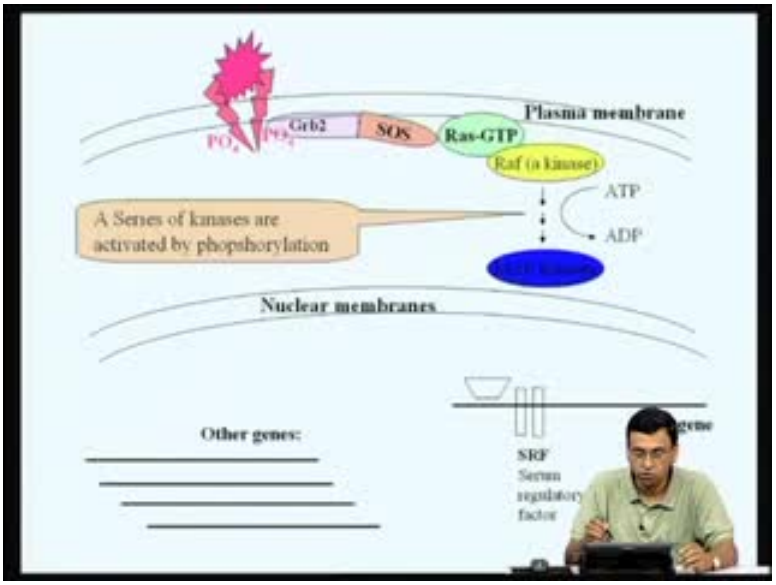

Now, map kinase now goes inside the nucleus and phosphorylates protein factor transcription factor likes the serum response regulatory factor or the SRF. Again we have discussed in detail, serum regulatory factor in previous classes when we were discussing about the GPCR. How again cell proliferation pathway can also be activated by G protein signaling involve in the GPCRs. And serum response factor is again a very important transcription factor which controls the expression of what is known as the immediate early genes. These are the genes which are immediately transcribed within a few minutes of addition of a serum two cells. For example, we know that for cells to proliferate mammalian cells, to proliferate in culture, you require serum and serum basically, contains the growth factors such as EGF, FGF and so on and so forth.

So, that is why cells to proliferate in vitro require serum. And serum through this growth factors ultimately activates the serum response factor. And this serum response factor now binds to what is called serum response elements in promoters of immediate early response genes such as c Fos and now, c Fos gene are now activated by RNA polymerase 2 and the c Fos messenger pre-messenger RNA is now synthesized, these messenger R N

A now goes to the cytoplasm gets translated to c Fos protein and since c Fos protein alone cannot form a homo dimer and cannot go on to bind to the target sequence, it requires hetero dimeric partner.

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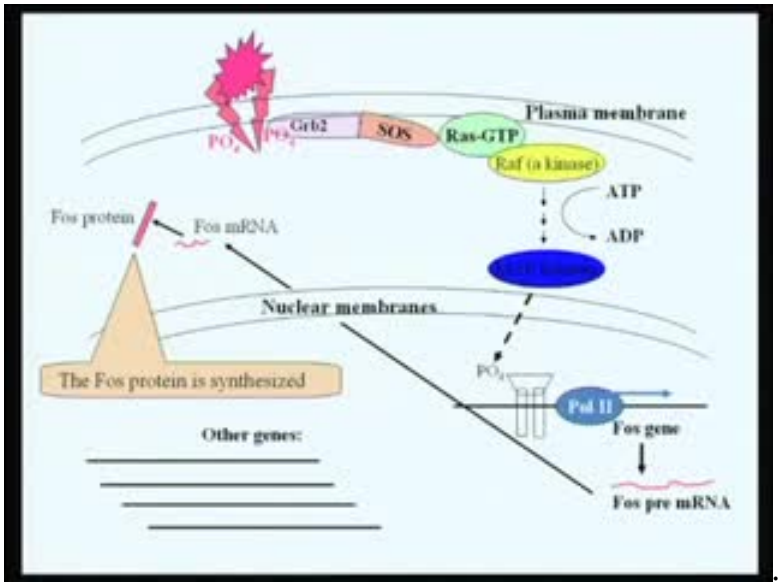

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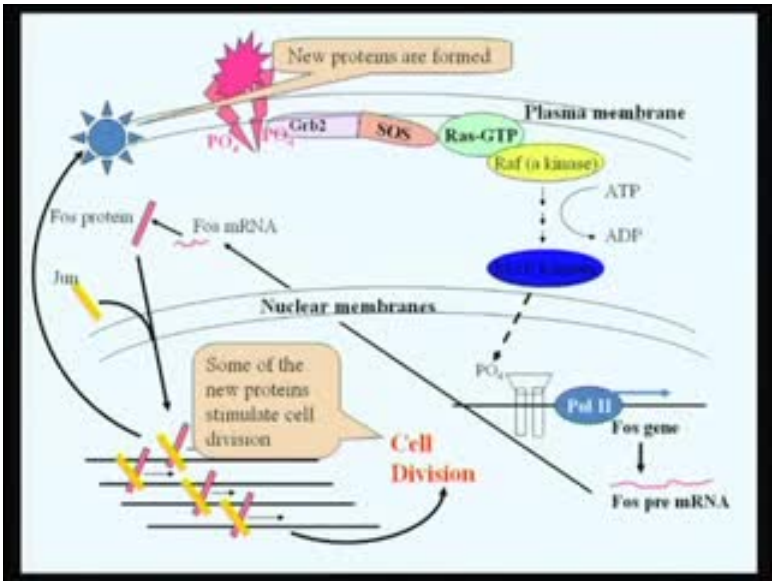

So, the c Fos protein now combines with Jun which photo form a Jun Fos hetero dimer and this Jun Fos hetero dimer goes and binds to what is known as the AP 1 response elements TGACTCA of again the late response genes growth promoting genes and this Juns which are now synthesize, now initiates cell division leading to cell proliferation

and mitoses and so on and so forth. So, this is basically the pathway by which a growth factor bind a growth factor receptor ultimately, ends in the activation of genes involved in cell proliferation.

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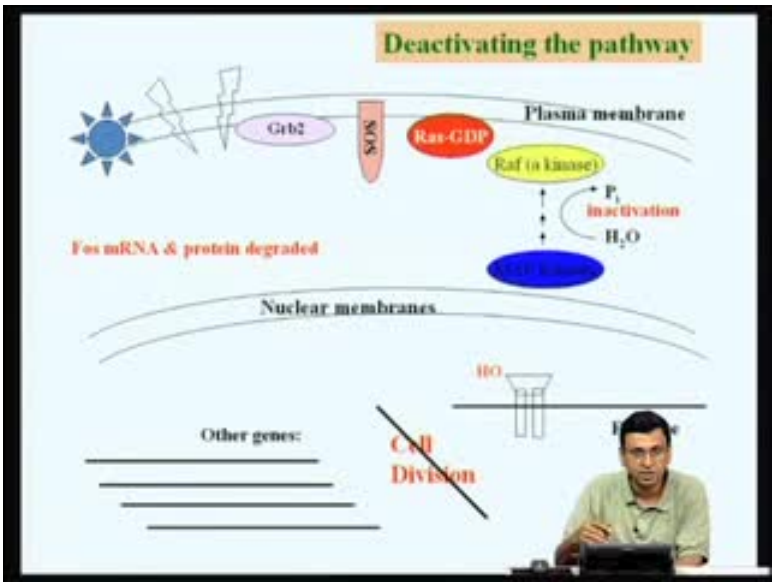

Now, once these events that are taking place the growth factor signaling also has to be switched off. So, what happens the normal cells? After a while the synthesis of the growth factor seizes and therefore, growth factor can no longer bind. Therefore, the receptor cannot dimerise, the receptors can becomes monomers and once the receptor become monomers because the tyrosines are not phosphorylated anymore. Grb 2 cannot interact with unphosphorylated form of receptor. And therefore, it neither interact through the SH 2 domain with the unphosphorylated tyrosine kinase nor it can interact with SOS through the SH 2 domain. And therefore, the Ras GTP can no longer remains now in the GDP bound form because SOS cannot exchange GDP to GTP.

So, Ras becomes inactive therefore, the map kinase cascade, map kinase are not activated and therefore, these genes transcript the serum response factor which is in the phosphorylated form is now dephosphorylated and it can no longer be phosphorylated by map kinases. Therefore, the Fos gene is not transcribed. Fos protein is not made. And Fos protein has a Fos mRNA has a very short half-life. So, the messenger which is already been synthesized is degraded. The Fos protein is also rapidly regarded. And as a

result there is no longer activation of these genes involved in cell division and cell proliferation. Therefore, cell division stops.

So, in the normal cells the levels of growth factors are very nicely maintained. Therefore, the growth factors go and bind the growth factor receptor only when necessary for a brief period, triggering the activation of the growth factor signaling pathway leading to cell proliferation. And once the growth factor synthesizes and the growth factors are rapidly degraded, the pathway is no longer activated. And therefore, the growth factor receptors cannot induce the activation of these target genes. And therefore, cell proliferation seizes.

Now, the problem happens in cancer cells is that when this regulation is disrupted at any of the stages, in cancer cell the regulation can happen either of the growth factor level or it can happen at the activation of the tyrosine kinase level or it can happen at the Ras activation or it can happen at the transcription level. Any abnormal event happening any of the stage of signal transduction results in the continuous activation of genes involved in cell proliferation leading to uncontrolled cell growth and cancer.

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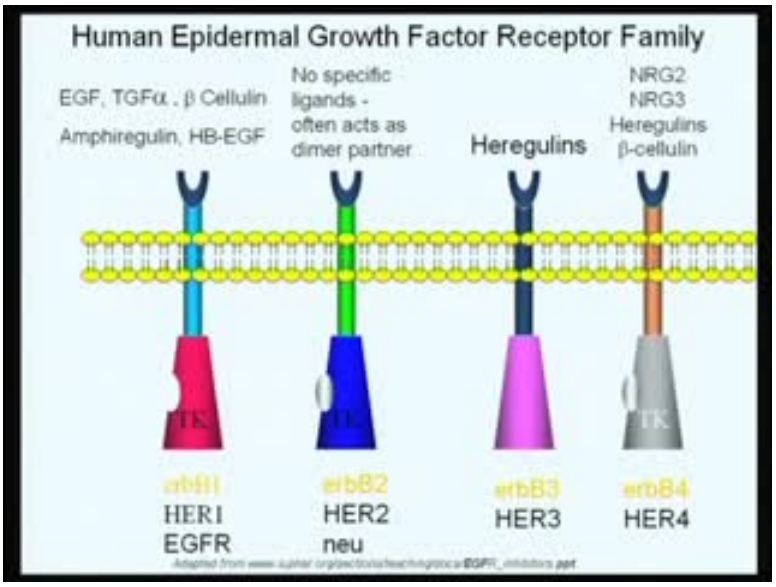

Now, I have given a very simplified version by which growth factors now activate, promotes cell proliferation by activating genes such as c Fos which are known as the immediate early response gene result in the activation of cell proliferation. But this is a very simplified version of the view the purpose is not to confuse you, but to understand

the basic aspect by which growth factors regulate gene expression or the growth factor signaling pathway is activated. Just to give an example, among the various growth factors which are listed earlier such as PDGF, FGF, PGF and so on and so forth. The epidermal growth factor itself, consists of a number of members in the epidermal growth factors family.

For example, the normal epidermal growth factor which are present in the normal cells is known as the epidermal growth factor receptor known as the erb B1 or HER 1 or EGF receptor, which is a normally present normal cells. It can bind to the growth factor and when the growth factor binds, it can form a homo dimers and results in the activation of tyrosine kinase domain and then leading to all the signal transduction that we discussed so far. Ultimately, adding to the activation repression of target genes. But, there are some of several variants of this growth factor receptor this normal growth factor receptor is bound by molecule such as EGF, TGF alpha, beta cellulin, HBEGF, amphiregulin and So on. Now, there are also several other versions for example, there is a variant of this epidermal growth factor receptor is known as the erb B 2 or HER 2 and this is present mostly in cancer cells.

Now, the erb B 2 differs from the erb B 1 since that it does not bind any specific ligands and it actually many cancer cells, it actually acts as dimeric partner. So, the erb B 1 instead of forming a homo dimers, it can actually form a hetero dimer with erb B 2 and this happens, it can lead to cancer. There also other members known as the erb B 3 or HER 3 for which molecules known as the heregulins actually bind, again I will not go into details because we are not really interested in discussing details about signal transduction pathways. I am just giving a examples to tell you how complicated this growth factor signaling pathways and even within just one growth factor in the EGF, there are so many variants, imagine there are so many such growth factors and so many variants have been reported.

And for example, there is another one called as the erb B 4 or H 4 again a different set of growth factors known as NRG 2, NRG 3, heregulins, beta cellulin they bind. Each of them bind to the receptors and this results in different physiological responses because they activate different cascading pathways. So, there are several variations in the EGF downstream signaling pathway. I have just listed some of the things, for example, when the epidermal growth factor binds to the epidermal growth factor receptor in the case of

erb B 1 or HER 1or EGF receptor, it actually results in the homo dimerization of the receptor and this results in the normal signaling pathway which have discussed so far.

Now, in case of cancer cells what happens the same EGF receptor can actually now hetero dimerise with the erb B 2 and when this happens, this hetero dimerization can actually lead to cancer. So, many of the cancers the erb B 1 instead of forming homo dimers, can actually form a hetero dimer with the erb B 2 and this hetero dimers can trigger uncontrolled cell proliferation leading to cancer. Similarly, there are many other different pathways like heregulins and others also activated receptors but we will not go into the details. So, just remember. So, variants of epidermal growth factor receptor which are expressed in different cell types depending upon forming different kinds of dimers, can being about variations of the signaling transduction pathway which are just discussed, leading to different kinds of physiological responses.

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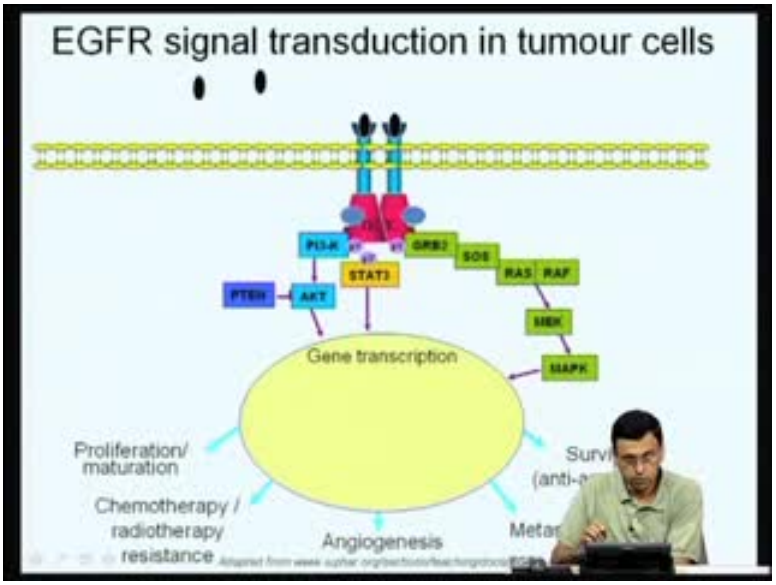

So, in the case of tumor cells what happens? The epidermal growth factor when it binds to the epidermal growth factor receptor and undergoes a specific dimerization, it can continuously activate either the Ras pathway or can also activate the phosphorylates kinase resulting in the pathway, known as the AKT pathway ultimately, resulting in transcription of genes involved in cell proliferation leading to cell proliferation, it can also lead to resistance of cancer cells to chemotherapy, radiotherapy and it can promote angiogenesis, it can also metastases and also can make cancer cell resistance for

apoptosis. So, in team each of receptor signaling transduction pathway in tumor cells is kind of slightly different from what happens in normal cells and this differential signaling pathway or the uncontrolled proliferation of this pathway can actually result in activation of multiple signaling pathways leading to uncontrolled cell proliferation and cell growth.

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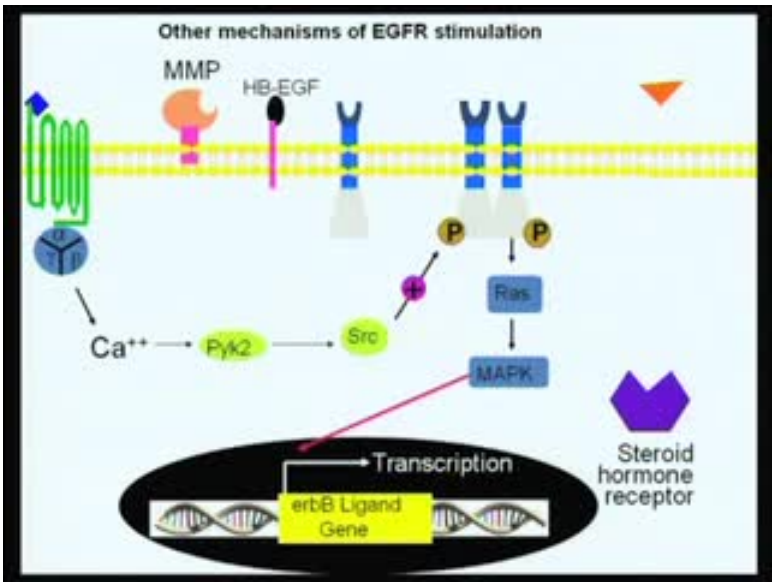

Now, I have indicated the point I am going to discuss now, that I have also indicated earlier when we were discussing about GPCRs, but these map kinases or this effector molecules like phospholipase C, protein kinase A or protein kinase C, they need not be to activate only by one pathway, but they can be activate by multiple pathways. So, in this cartoon I am going to just show, illustrate the same point where in, the same epidermal growth factor receptors can also be activated through G protein signaling. Here is a GPCR the 7 trans membrane protein and when they ligand for this G protein now binds, binding of this ligand to the GPCR can also trigger event, for example, when it activates a phospholipase C, that phospholipase C now results in the evasol phosphate, this phosphate into evasol triphosphate and protein kinase c. And protein kinase c now gets activated by diacylglycerol and the evasol triphosphate is now results in the release of calcium. This calcium through a different pathway, can go on phosphorylated tyrosine residue of growth factor receptors. And this tyrosine phosphorylation can then activate Ras pathway link map kinase activation and at transcription of the target genes.

So, you can see the receptor tyrosine kinases can be activated not only by the growth factor binding to the growth factor receptor, they can also be cross activated or if there is a cross talk with the G protein couple receptor pathway also. So, molecules or signaling molecules which bind to the G protein couple receptors can also activate the receptor tyrosine kinase through a different signaling mechanism. So, when this G protein gets activated and when this G alpha now, activates phospholipase C. Phospholipase C can clean phosphates, this phosphate resulting the generation diacylglycerol and calcium. Sorry, diacylglycerol and inositol triphosphate. The inositol triphosphate goes now in to Endoplasmic reticulum, resulting in the release calcium. This calcium can now activate Pyk 2, which in turn can activate Src and this Src can now go on phosphorylate the tyrosine residues and this phosphorylate tyrosine residues now, can activate Ras pathway leading to activation of gene expression.

There are also other mechanisms by which, for example, proteins known as matric metro proteinases can also release growth factors and this release of growth factors now, the growth factor can go on bind to the growth factor receptor promoting the dimerization resulting the activation of Ras pathway leading to activation of transcription. So, you can see the receptor tyrosine kinases can be activated not only by the growth factor directly bind to the growth factor receptors, they can also be activated through the G protein couple receptor pathway or they can also be activated by matric metro proteinases because the sequence of the growth factors on when the reusable factors now, the growth factors goes and binds and then activate.

Another mechanism by which this tyrosine receptor kinase activated is by steroid hormones. The steroid hormones can actually diffuse directly through the plasma membrane and when these hormones diffuse to the plasma membrane, they bind to intracellular steroid hormones receptors and when the steroid hormone receptor ligand complex now, can go and bind to the promote region of molecules genes coding for erb B 2 and now the erb B 2 gene gets transcribed and then get translated into erb B protein; erb B protein can now diffuse out of the cell, again come out bind to the growth factor receptors and then, induce again Ras activation leading to activation of pathway.

So, you can see the signal transduction pathways are not static. It is not that G protein couple receptors can activate only one particular pathway and receptor tyrosine kinase can be activated only by certain molecules. There is lot of cross talk among the signaling

pathways and a particular receptor or a particular signaling pathway can be activated through multiple channels. And I have given just one example here to show how a receptor tyrosine can be activated, either by matric metro proteinases or the G protein coupled receptors or directly by the growth factors or they can also be indirectly activated by steroid hormones. So, you can see that is why the entire signaling transduction pathways are very complicated and it is not as simple as I have shown in this schematic in the previous few slides.

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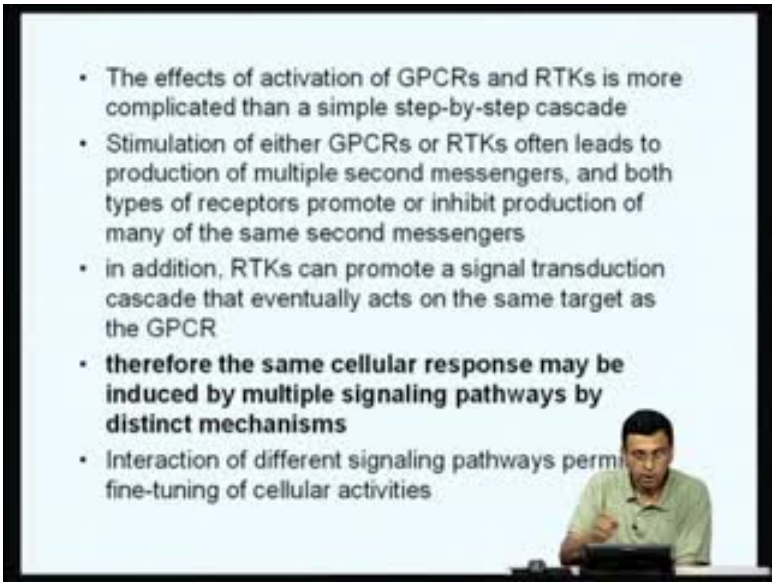

So, the effects of activation of GPCRs and receptor tyrosine kinases is more complicated than a step by step cascade, which we illustrated so far. In the last three classes we had studied GPCRs signaling separately now, we are discussing receptor tyrosine kinase signaling separately but, in the cell there is a lot of cross talk going on. Simultaneously activation of a GPR signaling pathway can also results in the activation of receptor tyrosine kinase pathway or when a growth factor binds to a growth factor receptor, it can also results in the activation of GPCR. So, there is lot of cross talk that is going on.

So, stimulation of either GPCRs or receptor tyrosine kinase often leads to production of multiple second messengers and both types of receptors promote or inhibit the production of many of the same second messengers. So, the same kind of second messengers like calcium or protein kinase A can also be activated by either of this pathways. Receptor tyrosine kinase can also promote a signal transduction cascade that

eventually acts on the same target as the GPCRs. So, the targets, the same target gene can ever be activated by either the GPS signaling pathway or it can also activated by the receptor tyrosine kinase pathway. For example, crept, crept can be activated either by GPS signaling or crept may be also activated by a particular growth factor, because the second messenger that is activated probably in this case is a protein kinase a cyclic AMP. If the cyclic AMP synthesis leads to second messenger synthesized by both these activations, it can phosphorylate protein kinase A and protein kinase A can now act on the crept and same kind of target genes can be activated.

So, the point I am going to make use that signal transduction pathways of pretty complex. This is not as straight forward as we have discussed so far. There is a lot of cross talk and multiple signaling can take place in the same cell and the one particular signaling molecule can activate multiple signaling pathways. The same cellular response may be induced by multiple signaling pathways by distinct mechanisms like, I just mention here. The crept transcription factors are can be activated either through GPCRs signaling pathway or it can be activated either by the receptor tyrosine kinase pathway or many other signaling pathway, that we are going to discuss in the next few classes. Interaction of different signaling pathways permits fine tuning of number of cellular activities. So, you can see the activation or repression of target genes can happen through multiple signaling pathways and there is lot of cross talk between these signaling pathways.

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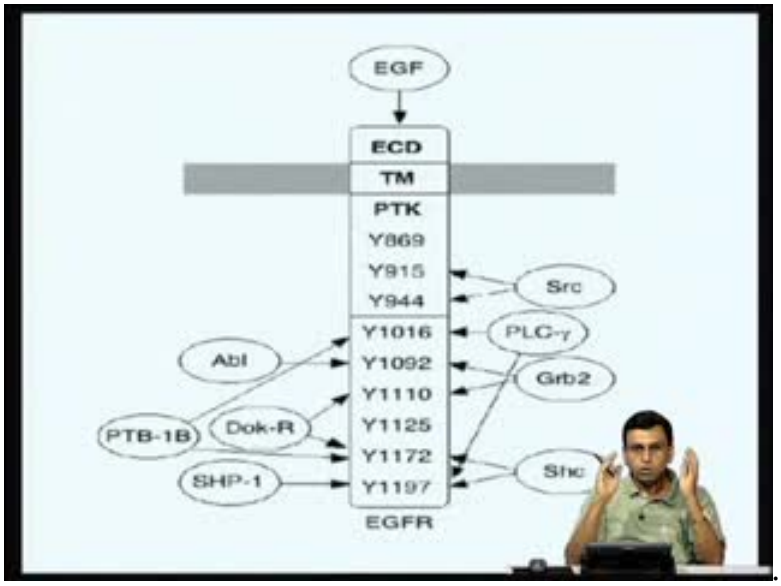

Now, the epidermal growth factor receptor that was studied So far, is one of the most well characterized membrane receptors every lot of things are known about it, the crystal structure is known, what kind of protein amino acids are phosphorylated, what kind of residues of everything is known, this cartoon just tells you the various tyrosine residues and what are the target proteins which interacts with specific phosphorylated tyrosines. For example, Grb 2 through its SH 2 domain, it actually interacts with tyrosine 1092 and tyrosine 1110 whereas, if the tyrosine 1016 of epidermal growth factor phosphorylated, it now interacts with phospholipase C whereas, if tyrosine 915 and tyrosine 944 is phosphorylated, it interacts with Src. So, you can see how complicated the whole scenario is. So, depending upon what kind of tyrosine residues are phosphorylated the effector molecules, the receptors interacting can be different and accordingly the signaling pathways are also pretty different.

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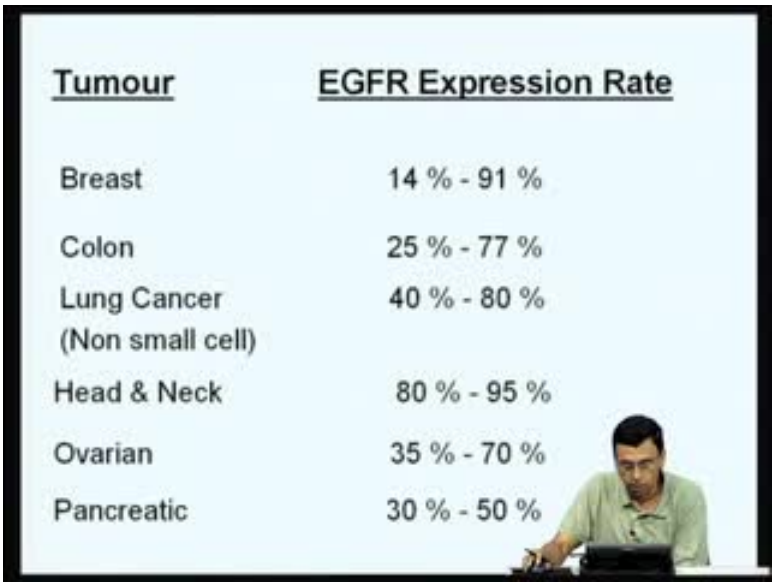

Now, why is this epidermal growth factor and growth factor receptor is so important. here I have just one example to show, how in different kinds of cancers like breast cancer, colon cancer, lung cancer, head and neck cancer, ovarian cancer, pancreatic cancer, in all these cancer you can see the epidermal growth factor are highly over expressed. In for example, breast cancer the expression rate of EGF receptor is 14 percent to 91 percent above that express the normal cells. So, the epidermal growth factor express very high levels in a way of these cancer cells because basically the cancers cells want to proliferate continuously therefore, by over expressing growth factor

receptors, it can be continuously bound by the growth factors and as a result the growth factor receptor pathway can be continuously activated resulting in continuous activation of the genes like c Fos leading to continuous cell proliferation. So, I can see in a number of cancers, the expression rate of the growth factors are pretty high. And this is the one of the reasons why these cancer cells proliferate uncontrollably.

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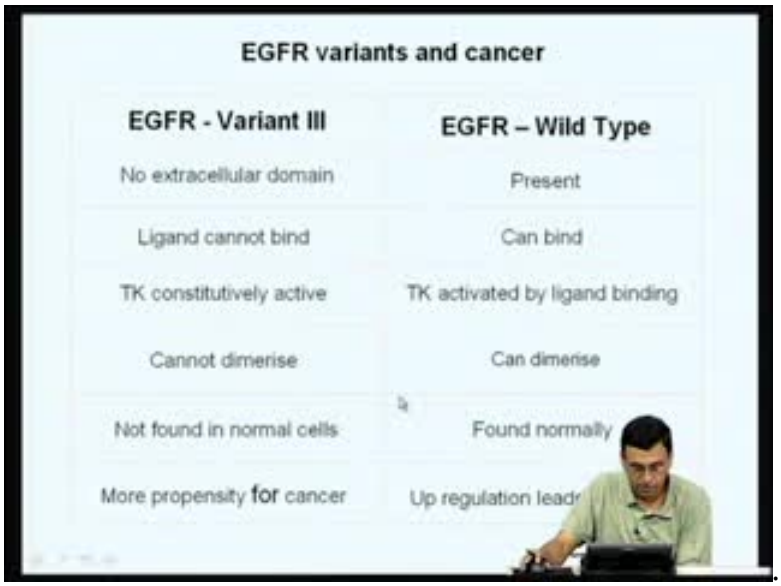

There are also number of variants of epidermal growth factor receptor detect number of cancer cells for example, there is one particular variant of epidermal growth factor receptor which has been shown to be expression of cancer cell which does not have an extracellular domain. Therefore, it cannot bind to the growth factor as I shown whereas, the normal receptor can bind. And this variant therefore, the tyrosine kinase remains constitutively active. Therefore, it continuously interact with Grb 2, continuously activating Ras, resulting the continuously activation of map kinase pathway and continuous activation of the genes involved in cell proliferation. And this variant also cannot dimerise. And therefore, it actually cannot be regulated and these variants such as what we discussed here is normally found in cancer cell but not in normal cells and such cells which express this kind of EGF receptor variants are highly susceptible to become cancerous.

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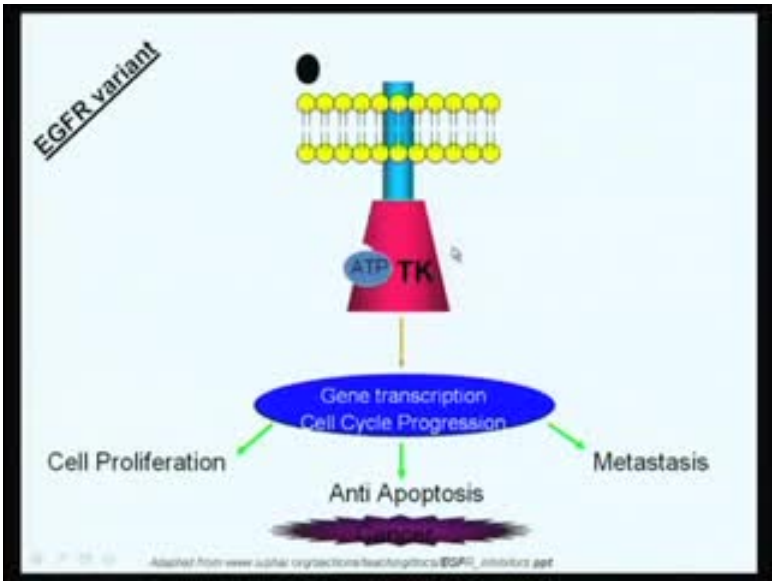

This is the cartoon that actually tells you that what I have just described here. This particular variant you have three, does not have extra cellular domain therefore, the epidermal growth factor cannot bind to the extra cellular domain. The tyrosine kinase domain is constitutively active therefore, using ATP it can get trans phosphorylate and it also get undergoes dimerization. Therefore, it gets continuously phosphorylate on tyrosine residue and as a result the signal transduction pathway is continuously on and therefore, there is continuous cell proliferation anti apoptosis, metastasis, which are all the hallmark of cancer cells. So, number of cancer cells express variants of this epidermal growth factor receptors which have by certain mutations have lost ability to bind ligand and therefore, the tyrosine kinase domain is continuously activated leading to uncontrolled cell proliferation because of activation of the genes involved in the cell proliferation.

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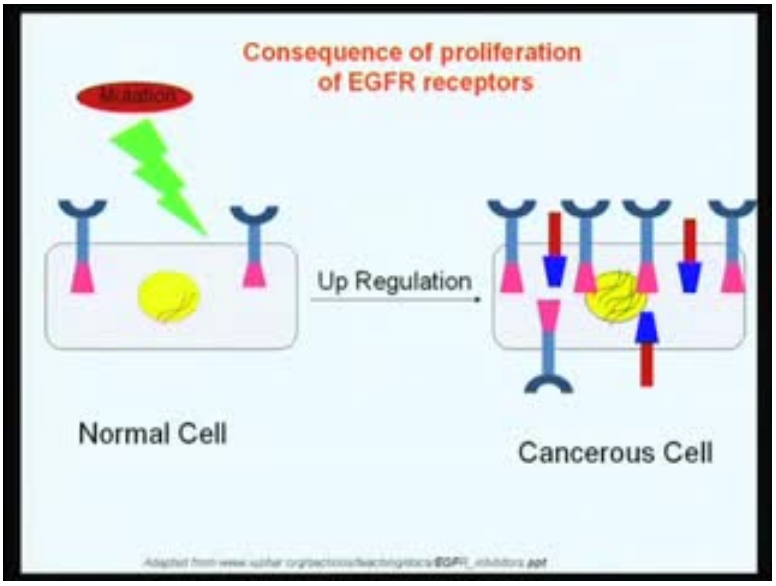

So, the proliferation of EGF receptor, you can see in the normal cell the level of expression of the EGF response cell surface is very finely regulated. You only find a few receptors on the cell surface whereas, in the case of cancer cells because of certain specific mutations, you can see the cancer cells, there is either up regulation the number of receptor cells have increased or there are certain variants of the receptors have been made. For example, the receptor which have extra cellular domain and therefore, they cannot dimerise and therefore, they can be constitutively activated and all these events ultimately lead to uncontrolled cell proliferation and cancer. So, a number of cancers either over expression of epidermal growth factor receptor or expression of variants of growth factor receptors is primarily responsible for a normal cell becoming a cancer cell.

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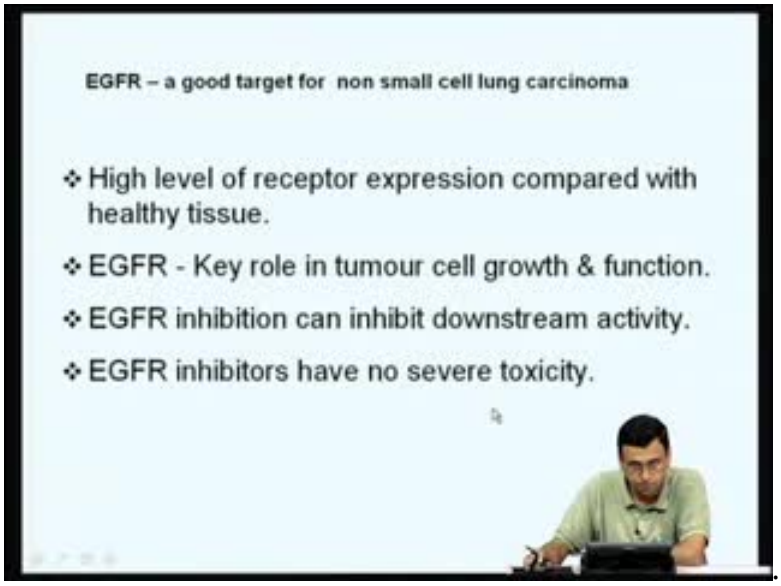

Because of this particular observation that epidermal growth factor receptor, there is a very good correlation between the levels or variations of epidermal growth factor receptor and cancer. The epidermal growth factor receptor has become an excellent target for cancer. So, a number of approaches to kill cancer cells are to treat cancer, primarily revolves around epidermal growth factor receptor and I will just give you a one or two examples. For example, just in the previous slides I had shown the non-small cell lung carcinoma, the epidermal growth factor is highly over expressed. Therefore, in that such kind of cancers the epidermal growth factor receptor is a very attractive candidate for cancer therapy. The high level of receptor expression compared with healthy tissue, the epidermal growth factor receptor is a key role in tumor cellular growth and function in the case of the lung carcinoma. If you inhibit a epidermal growth factor receptor in this cancer cells, you can effectively inhibit all the downstream signaling and therefore, you can stop proliferation of the cancer cell and if you now, these such kind of inhibitors which inhibits the epidermal growth factor receptor in the cancer cells, they do not have very high levels of toxics in the case of normal cells.

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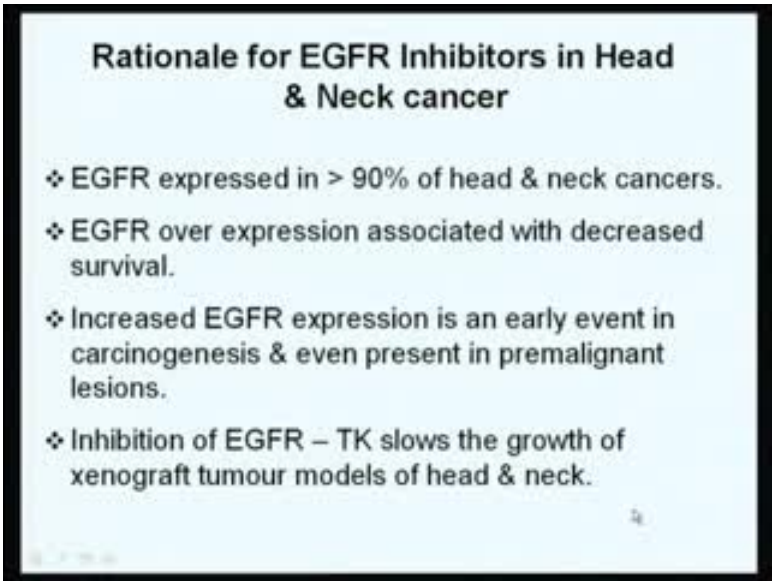

So, similarly in the case of head and neck cancer again, the epidermal growth factor receptor inhibits are being tried as a cure for head and neck cancer. Again as you can see the EGF receptor is over expressed is almost 90 percent of the head and neck cancers and this over expression associated with cancer and decrease survival. And increased EGF receptor expression is an early event in carcinogenesis and even present in premalignant lesions, that is even before the cells become malignant, you can see over expression of epidermal growth factor receptors. So, if you can block you can actually prevent the cancer become a malignant. So, inhibition of the growth factor receptor tyrosine kinase, slows the growth of the xenograft tumors models of head and neck. So, numbers of experiments are actually shown, if you can inhibit the epidermal growth factor receptor signaling in these head and neck cancer cells, you can prevent tumor growth.

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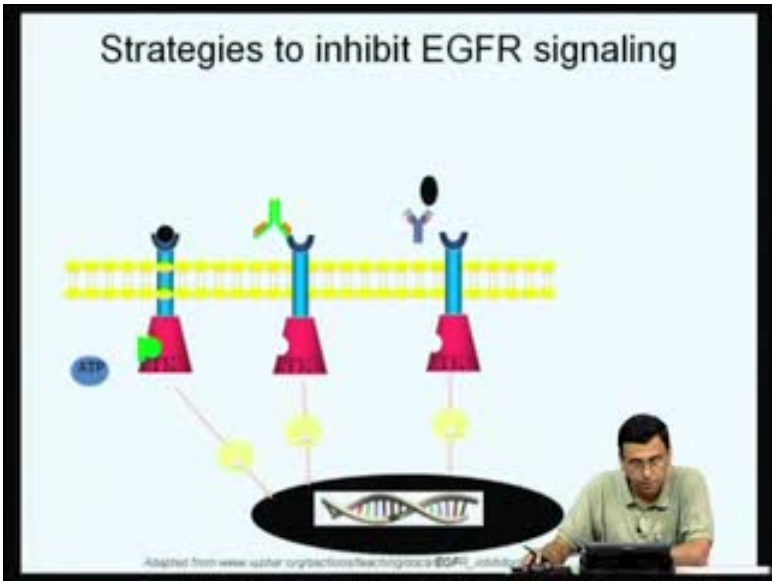

So, let us now try to understand what are the various strategies that are been devised to inhibit epidermal growth factor signaling. What kind of molecules have been designed? One approach is to design molecules which actually act as inhibiters of the receptor tyrosine kinase. As I said the receptor tyrosine kinase to be active, it has no bind to ATP. Now, if you can develop a decoy which should now go and bind to the ATP binding set of tyrosine kinase therefore, ATP molecule can no longer bind and therefore, the receptor tyrosine kinase cannot be active. And therefore, one approach of treating cancer is to develop molecules which are very efficient, inhibitors of tyrosine kinase and therefore, the tyrosine kinase can no longer be active and therefore, genes involved in cell proliferation cannot be activated. Therefore, cell proliferation can be proliferation of cancer cell can be block. The other approach is to devise molecules like monoclonal antibodies which go and bind to the extra cellular domain of the growth factor receptor. So, when these monoclonal antibodies go and bind to the extra cellular domain, the epidermal growth factor cannot bind and therefore, a signaling does not takes place.

So, only when the epidermal growth factor binds to the extra cellular domain, it undergoes a conformational change, so that it can dimerise. Whereas a monoclonal antibody binds a such kind of a conformation does not takes place therefore, the receptor cannot dimerise and therefore, downstream signaling does not takes place and therefore, gene expression can be blocked. The other way is to devise more antibodies which actually go and bind to the epidermal growth factor itself. So, when these antibodies go

and bind epidermal growth factor itself, a growth factor cannot bind to the growth factor receptor and therefore, the signaling is block and therefore, you can stop the propagation of cancer cells.

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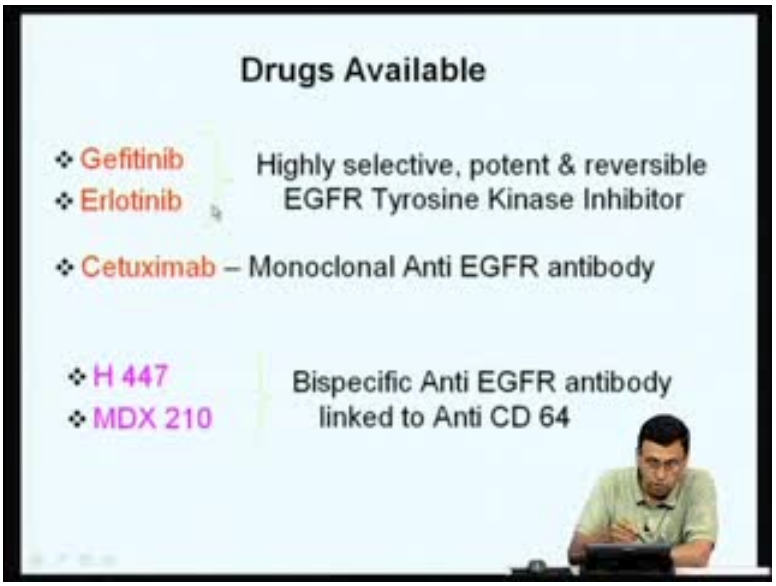

So, there are number of such approaches. So, based on these kind of approaches a number of drug molecules have been developed and you can see, these are now available as Gefitinib, Erlotinib, Cetuximab, either these are all approved for human use or there are under various stages of (( )) trial and this molecules are highly selective and potent reversible, EGF receptor tyrosine kinase inhibitors. So, they go and bind to the ATP binding domain of the tyrosine kinase. Therefore, the enzyme activity is blocked. Cetuximab is actually a monoclonal antibodies against the EGF receptor. Therefore, growth factor cannot be bind, EGF cannot bind and therefore, the signaling is blocked and there also many other approaches. Also a number of approaches are being used to block the EGF receptor signaling, so that genes involved in cell proliferation cannot be activated and therefore, cancer cell proliferation can be stopped.

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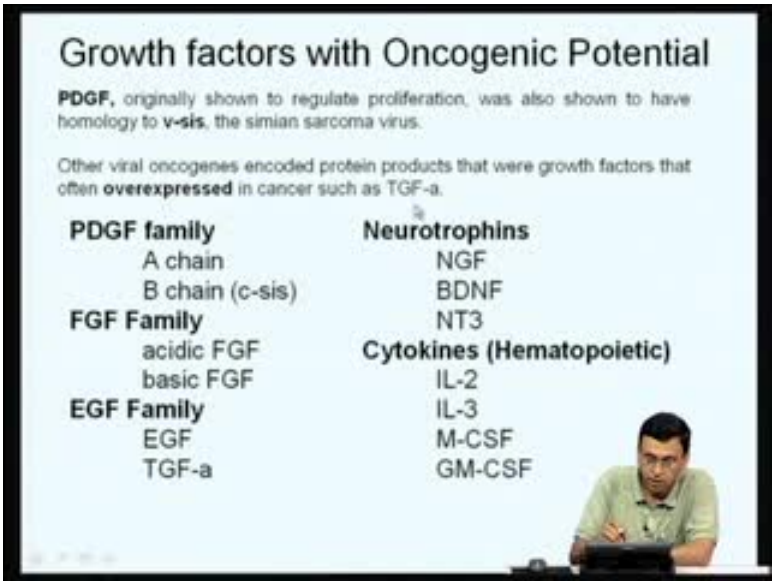

So, just like EGF there also a number of other growth factors which have been associated with cancer cells, mutations or variations of this growth factors have been shown to be present a number of other cancer cells and as a result, all these growth factors are also targets for (( )). For example, PDGF originally shown to regulate cell proliferation also has homology to a very important viral oncoprotein called the v-sis. So, This v-sis is actually has such notations PDGF and as a result this virus infects, the cell proliferation program is continuously activated and therefore, it becomes cells, become oncogenic and then gets transformed and then leading to cancer.

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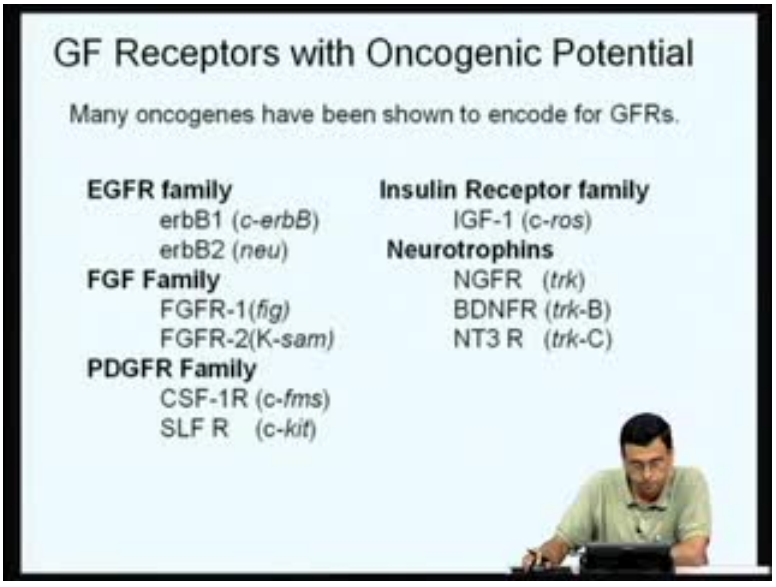

Similarly, there are certain cancer cells in which the TGF alpha is highly over expressed as a result, it results in cancer. So, basically this list of actually shows that a number of growth factor receptors have a oncogenic potentials and many oncogenes have been shown to encode variants of growth factor receptors. I have just given the some of this variants in the parenthesis and many of this growth factor receptors either the EGF receptor family, the FGF family or the PDGFR receptor family and so on and so forth. They are all either variants mutant forms of this receptors or express in cancer cells or there all present in the viruses and therefore, they can result in cancer.

So, I think what I have discussed so far here is a very important signal transaction pathway namely, the growth factor and growth factor receptor signaling. and I have discussed very briefly how when a growth factor binds to a growth factor receptor, how the tyrosine kinase activity of receptor is activated, ultimately leading to the activation of a Ras and Ras now results the activation of map kinases and map kinase now, go and actives specific transcription factors which in turn activates genes involved in cell proliferation such as c Fos, leading to cell proliferation. And if this regulation of this pathway is lost it results in uncontrolled cell proliferation leading to cancer.

So, what I have listed here is a number of important online resources that we can make use of. I myself made use of many of these online resources. One can go to the many of this websites and look at this websites and see you can get a number of very important information on growth factor signaling. So, one can just copy and paste any of this websites, web links I have given here and then go to these websites and you can get a wonderful illustration of growth factor signaling and many of the cartoons I have shown in this are also taken from some of these websites basically to explain how growth factor signal is very important.

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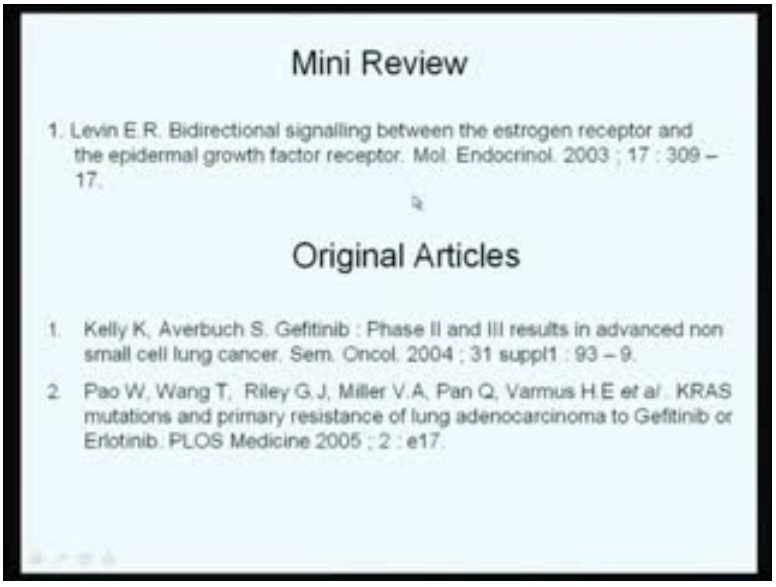

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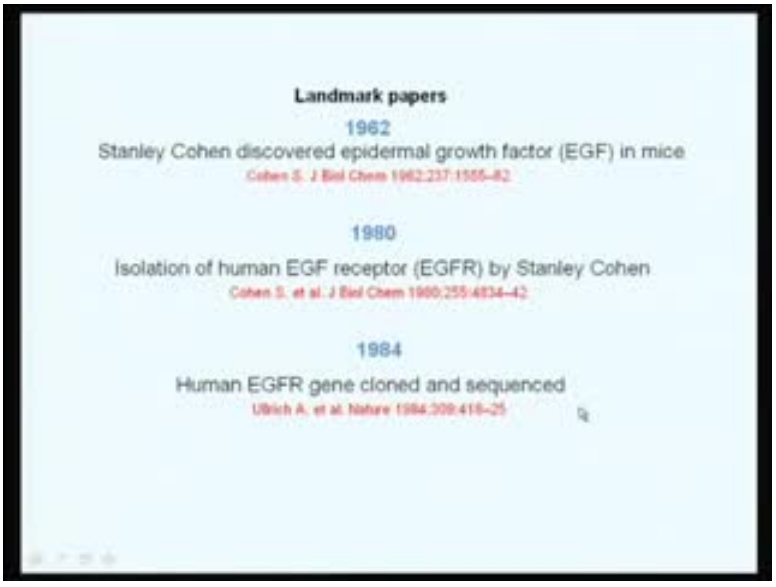

I have also listed a number of review articles here. One can go through this review articles and then get more knowledge level growth factor signaling. there also many reviews, there are original research articles and last but not least, I have actually listed here the two class review articles written by the original discovers of growth factors namely, the Stanley Cohen and Rita Levi-Montalcini are very nice reviews in JBC in 2008 an scientific American. And one can go through this to understand how exactly the growth factors signaling is very important.

And finally, I have listed the three landmark papers that I have let to discovery of growth factors and growth factor receptors, published in JBCs nature namely, the discovery of epidermal growth factor, the isolation of epidermal growth factor receptor and the cloning of the human epidermal growth factor receptors. Some of these are landmark papers that actually change the entire growth factor signaling pathways. I think, I will stop here.