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Signal transduction in cancer

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Abstract
Cancer is characterized as abnormal growth in cells which have managed to evade central endogenous control systems. This abnormality can arise from either genetic or epigenetic change. A cascade of signaling pathways are responsible for cell growth, division and death. Dysfunctioning in these pathway leads to overexpression or underexpression of some molecules fuel cancer proliferation. Due to rapid technological advances in last few years, it is possible to analyze the signaling pathways involved in cancer proliferation and therefore simpler diagnostic in a shorter period of time. PI3K-Akt and Ras-EKT pathways are two major pathways which are to be examined for cancer treatment.

Keywords: Signal transduction, cancer, cell growth

Introduction
Throughout our lives, healthy cells in our body divide and replace damaged cells in a controlled manner. Genetic changes such as mutations and epigenetic changes causing activation of oncogenes and inactivation of tumor suppressor genes leads to uncontrolled cells division and growth. Loss of apoptosis, metastasis and angiogenesis are three characteristic alteration developed by cancer cells. Apoptosis is a cell death mechanism for malfunctioned cell. Cancer cells evades apoptosis by various mechanism such as inactivation of death induced signaling complex, reduced level of proapoptotic complex, upregulation of inhibitors of apoptosis. Metastasis is defined as spread of cancer cells to new areas of body; mainly through extracellular matrix (ECM). The key player in this process is enzyme matrix metalloproteinase (MMP). Not only has it helped in breaking of ECM proteins but also cleavage of Fas ligand thus blocking apoptosis. Though most cancer cells are malignant, but some cancer cells are benign in nature i.e. do not spread in other parts of the body and do not create new tumors. After expansion of tumor, these cells run short of oxygen and other nutrients which are provided by formation of new blood vessels i.e. angiogenesis. The most important role in angiogenesis is played by vascular endothelial growth factor and its receptors. These series of complex changes in our body leads to dominant growth of cancer. Also there are some pathways which play an important role in proliferation of cancer i.e. Ras-ERK pathway and PI3K-Akt pathway. Here we will be describing signaling in both the pathways and its changes which leads to cancer development. Also we will emphasize on genes, receptors, proteins and molecules which plays role in signal transduction of cancer.
In normal cells, gene expression and signal transduction are complementary to each other. So we can also link genetic alterations with abnormal signal transduction which is the onset of cancer growth and development. Ligand binds to the extracellular receptor domain, generally receptor tyrosine kinase (intracellular receptor domain) which activates a cascade of signals which goes to nucleus. Also cell surface receptor (CSR) production is limited by cellular restrains on gene expression and protein translation. Any mutations in gene encoding receptor disrupt this finely tuned regulation. Thus producing too many copies of gene; a phenomenon called gene amplification. This results in overexpression of receptors which causes Ligand-independent signaling. Extracellular growth factor receptor (example: HER1 or cErbB-1) are type 1 molecules of receptor tyrosine kinase (RTK) which play significant role in regulation of cell proliferation, differentiation and survival. The end result of any growth factor receptor signal transduction is mitosis. If mitosis continues, there is exponential growth potential of tumor cells.

Loss of Apoptosis

Apoptosis is a form of programmed cell death. It is characterizes by cell shrinkage, mitochondrial cytochrome c release, fragmentation of cell DNA and cell breakage. There are two pathways to activate apoptosis- extrinsic and intrinsic. Apoptosis begins with extrinsic pathway or the death receptor pathway. Fas and Fas Ligand are members of tumor necrosis factor (TNF) receptor and TNF family, respectively. When they ligate, FADD (fas associated protein with death domain) which recruits apoptotic pro-caspase to form death-inducing signal complex (DISC) such as caspase 8 followed by activation of caspase 3, is activated. If DNA damage is caused by external means like UV radiations, viruses and chemicals, then intrinsic pathway or mitochondrial pathway comes into role for apoptosis. p53 gene gets activated which acts as a transcription factor and activates BAX or BAK gene. BAX and BAK are proapoptotic factors which stimulate release of cytochrome c from mitochondrial membrane. Cytochrome c along with APAF1 (apoptotic protease activating factor 1) activates caspase 9 which in turn activates caspase 3. Both these pathways stimulate a set of enzymes called caspases (cysteine-aspartic proteases) with an ultimate motive to activate caspase 3 which is the executioner caspase in apoptosis. Active caspase 3 degrades multicellular proteins, and is responsible for morphological changes and DNA fragmentation during apoptosis.

Malignant cells are able to resistance to apoptosis due to overexpression of anti-apoptotic proteins such as IAP proteins, Bcl-x and Bcl-2 family of proteins. They bind to proapoptotic proteins such as BAX and BAK thus blocking apoptosis. Also, activation of transcription factors can lead to apoptosis resistance. For example, when the members of nuclear factor- kappa B, which are important for p53 mediated cell- death, are overexpressed in certain tumor leads to increased level of transcription in IAP and Bcl-2 families. Tumors cells also evade apoptosis by reducing levels of CD95 (fas receptor), inhibition of death induced signaling complex (DISC) by FLICE protein, reduced production of cytochrome from mitochondria as a result of upregulation of Bcl-2, reduced levels of pro-apoptotic BAX resulting from loss of p53, loss of APAF1 (apoptotic protease activating factor 1 and upregulation of inhibitors of apoptosis protein (IAP) which prevents activation of caspase 9. Tumor cells are able to survive as they escape apoptosis and multiply, rigorously. Also tumor cells are able to proceed to next stages of cancer progression i.e. metastasis and angiogenesis.

Metastasis

The spread of cancer cells from the site where they first formed to another part of body is known as metastasis. Cancer cells originates and breaks from the original (primary) tumor, travels via the lymph or blood vessels and form new tumor in other organs or tissues in body. It involves interaction between tumor cells, lymph vessels, basement membrane and extracellular matrix (ECM). Within the circulation, tumor cells tend to aggregate in clumps which are either heterotypic i.e. clumping of tumor cells and blood vessels or homotypic i.e. clumping of only tumor cells. Migration of tumor cells can occur through two processes- through matrix metalloprotenase (MMP) or amoeboid migration. There are basically two steps in metastasis- invasion of ECM and vascular dissemination, homing of tumor cells and their colonization. Cell activation process involves production of enzyme MMP which degrades collagen of ECM. It allows tumor cells to migrate towards blood vessels through tight junctions of epithelial cells. Cancer and epithelial cells forms weak adhesion between them which allows cancer cells to easily roll over the epithelial lining. Later cancer cells leave blood vessels and enter tissue. They also create gap in epithelial lining to allow less aggressive tumor cell to enter tissue and grow. Another mechanism by with tumor cells migrate is by squeezing in of cancer cells through spaces in matrix instead of cutting its way through. This is called amoeboid migration. Only malignant tumors can metastasize. Uncontrolled cell division (Primary growth) is observed in all kinds of cancers followed by angiogenesis to provide nutrients to tumor cells. Development of blood vessels paves way towards blood stream for malignant cells and becomes mobile. Thereafter, these cells invade in other tissue through blood stream by breaking ECM. Also they are able to survive during systematic circulation. Some malignant cells become dormant whereas some are able to form secondary tumors. Ras-ERK and akt/PI3K/mTOR pathways are able to regulated metastasis by multiple downstream regulators like Rho-family GTPases, integrins and matrix adhesion proteins, extracellular proteases which degrade ECM, cell-cell adhesion complexes that affect matrix adhesion strength and Transcription factors such as AP1 and Ets2 that regulate expression of many proteins that control migration/polarity, including matrix metalloproteinases (MMPs), plasminogen activator, cadherins, and actin regulators.

Dysfunctioning of Regulatory Pathways

Ras-ERK and PI3K-Akt pathway are important regulators of normal cell proliferation. Ras-ERK pathway plays an important role in mitogen activated protein kinase (MAPK) cascades and is the most important signaling pathway among all MAPK signal transduction pathways. Ras protein is an intracellular G protein present below plasma membrane. It has generally two conformations- an activated GTP conformation and an inactivated GDP conformation. Ras protein can oscillate between these two conformations to regulate signal transduction. Ras is activated by many stimulating factors, such as epidermal growth factor (EGF), tumor necrosis factor, src family and activators of protein kinase C. When extracellular signals interact with receptor, growth factor binding protein2 (grb2) binds to proline rich sequence at C terminus of son of sevenless (SOS). When SOS interacts with tyrosine phosphorylation site on receptor, it gets translocated to membrane from cytoplasm. It creates high concentration of SOS near ras following which GDP is replaced with GTP in ras. This cascade of interaction activates ras protein. Mutated ras is unable to hydrolyze GTP back to GDP. So it becomes permanently active thus overproducing cell growth proteins like cyclins and CDKs. Another protein involved in ras-ERK pathway is raf protein which exhibit serine/threonine kinase activity after binding to ras protein. B-raf shows the strongest activity among all subtypes but also has highest mutation rate which is 90% in melanoma. Activated raf interacts with MEK (MAPK/ERK kinase) which activates MEK. MEK is a rare dual-specificity kinase that activates ERK (extracellular signal- regulated kinase) by phosphorylating the Tyr and Thr regulatory sites. Also it anchors ERK to cytoplasm. Once the signal stimulates phosphorylation and dimerization of ERK, it gets activated and is translocated to nucleus. There ERK regulates other protein kinases followed by further phosphorylation of downstream substrates. In nucleus, the phosphorylation of nuclear transcription factors such as proto-

oncogene c-Fos, proto-oncogene c-Jun, ETS domain-containing protein Elk-1, takes place and induce gene expression in response to external stimuli. These genes makes protein those are important for cell growth, survival and activity. Continuous activation of ras-ERK pathway can transform normal cells into cancer cells. Elevated expression of ERK is seen in almost all types of human cancer. One of the important targets of this pathway is transcription factor myc. Myc gene encodes transcription factor myc which makes other proteins like CDKs, E2F family and plays important role in cell growth, survival and activity.

Another pathway involved in cell fate determination is PI3K/AKT/mTOR pathway. PI3K (phosphatidylinasitide-3-kinase), AKT and mTOR are the main components of PI3K/AKT/mTOR pathway. PI3K are family of enzymes that are involved in cellular functions like cell growth, differentiation, motility etc. They are recruited by the activation of RTK and GPCR by growth factor. This stimulates the level of PIP3 (phosphoinositol triphosphate). PIP3 acts as a secondary messenger, facilitating the recruitment and activation of kinases that possess the pleckstrin homology (PH) domain, which can bind phosphatidylinositol lipids within biological membranes, such as PI3K-dependent kinase-1 (PDK1). Signaling duration in PI3K is regulated by phosphatase and tensing homolog (PTEN), which acts as inhibitors of PI3K activity. Along with PDK1, PH domain of protein kinase B (PKB), also known as ser/thr kinase AKT, is recruited to plasma membrane. Following activation, AKT inhibits tumor complex 1/2 (TSC 1/2) by phosphorylation. TSC1/2 is a negative regulator of mTOR1. It inhibits rheb which is an activator of mTORC 1. This then phosphorylates eIF4-binding protein, releasing the eIF4E capbinding factor and allowing it to bind mRNAs, and p70 RSK (ribosomal S6 kinase). This promotes increased protein synthesis which is important for cell cycle progression. AKT also inhibits FoxO (fork head box O) transcription factor, which affects cell fate. It regulates cell fate by inhibiting cell survival and proliferation. Not only, can Akt regulate several enzymes involved in the G2/M transition but also can phosphorylate and inhibit proapoptotic proteins to prevent apoptosis. Thus overexpression of akt itself is the cause of many cancers. Also alteration in any node of akt/PI3K/mTOR pathway has been observed in many types of cancer.

Angiogenesis

As the tumor grows, it requires additional vasculature. They secrete proteins which stimulate blood vessel growth by process called angiogenesis. Pathway involves vascular endothelial growth factor (VEGF). It affects endothelial cells that line blood vessels. VEGF causes them to activate MAP kinase signal transduction pathway or induce proteins that breaks the basement membrane to allow endothelial cells to invade. Proteins involved are MMP, uPA (urokinase type plasminogenic activator) and its receptor uPAR and plasminogen activator. In tumor cells, when ECM is broken by MMP, proangiogenic receptor including VEGF can reach receptor on endothelial cells of blood vessels surrounding the tumor thus stimulating the angiogenic signals in vessels. VEGF also keeps new endothelial cells to survive by upregulating apoptotic inhibitors. Also, it activates endothelial cells to express proteins necessary to allow new blood vessels to form.

VEGF (especially VEGF-A) binds to VEGFR (especially VEGFR2). These receptors are RTK which causes dimerization and autophosphorylation. VRAP (signaling molecule) is activated along with other proteins required for cell survival and permeability. Another response is activation of PLC- gamma (phospholipase C gamma) which release calcium, activating protein kinase C (PKC). This cascade leads to cellular proliferation of endothelial cells. Also VEGF activates proteins likes NCK (activates MAPK) which takes part in nucleus signaling and SHB which is responsible for cell migration and adhesion of endothelial cells. Undoubtedly, VEGF stimulate the formation of blood of blood vessels and its overexpression is the major cause of angiogenesis.

PI3K/akt/mTOR and ras-ERK pathway also induces angiogenesis. Synthesis and secretion of VEGF by cancer cells is induced by HIF1 (hypoxia induced factor 1. PI3K/akt signaling increases HIF1 levels. Thus, hyperactivation of this pathway leads to induction of angiogenesis. Also, PI3K-Akt pathway also modulates the production of other angiogenic factors, such as nitric oxide and angiopoietins whereas, ras-ERK pathway activate transcription factor to enhance VEGF transcription.

Another factor for angiogenesis is thrombospondin1 (Tsp1) which are inhibitory proteins secreted by various cells which that regulates angiogenesis by inducing synthesis of FasL, which causes endothelial cells to undergo apoptosis.Tsp1 is induced by p53, but repressed by Ras, Src, and Myc. It thus represents another control point in angiogenesis that could be activated by deregulation of the Ras-ERK pathway. In some cancers, it is found that the gene encoding Tsp1 is mutated.

Genes related to Cancer Signalling

There are plethoras of factors that act throughout the life of a cell. It may be growth factors like growth factor receptor, transcription factors, signal transduction proteins, non-receptor protein kinases, cell cycle control proteins and regulators of apoptosis. These factors are controlled by number of genes. Mainly three types of genes whose alterations causes' tumor formation, exists. First type is of gene that turns healthy genes to cancerous gene by its activation. They are called oncogenes. Two common oncogenes are- The RAS family of genes, which makes proteins involved in cell communication pathways, cell growth, and cell death and HER2 that makes a specialized protein that controls cancer growth and spread. Another type of cancer related gene is DNA repair gene. These fix mistakes made when DNA is copied. Many of them function as tumor suppressor genes. BRCA1, BRCA2 and p53 genes are DNA repair genes. If mutations occur in these DNA repair genes then they cannot be rectified. These mistakes become mutations which causes cancer. Third type is tumor suppressor gene. They limit cell growth by regulating cell proliferation, death and survival. Cells grow uncontrollably when tumor suppressor genes are mutated and they may eventually form a tumor. Examples of tumor suppressor genes are p53, BRCA1 and BRCA2. The most common mutated gene in cancer is p53 or TP53. Generally p53 is missing or defected in cancer. P53 helps in growth arrest, apoptosis, inhibition of angiogenesis and DNA repair.

When a DNA is damaged, ATM/ATR/MDM are activated which are protein kinases that leads to p53 activation. P53 acts as a transcription factor to produce proteins for cell arrest like p21 and 14-3-3-δ. p21 binds to cyclin E-CDK2 and blocks phosphorylation airway protein which is required for entry into S phase of mitosis. 14-3-3-δ binds to CDC25c. This

complex localizes in cytoplasm where it cannot act upon CDC2. Block at this checkpoint occurs through inhibition of cyclin B. Another example of tumor suppressor gene is BRCA (breast cancer gene). Two types of BRCA genes are found to cause breast cancer in humans- BRCA1 and BRCA2. Both these genes contribute to DNA repair and transcriptional regulation in response to DN damage.

Ras genes, as discussed earlier, increase cell proliferation, reduce apoptosis and increase angiogenesis. Single point mutations in these genes induce oncogenic transformation, resulting in a continuously activated ras protein. Human epidermal growth factor receptor 2 (HER2) is a growth promoting protein on outside of all breast cells. Amplification of HER2 causes progression of aggressive type of breast cancer.

Conclusion

We can conclude that cancer is result of mutations in growth control gene resulting in disruption of molecules in signaling pathways like PI3K/Akt/mTOR and ras-ERK pathways. Given the cross-talk between pathways and robustness of cancer network, it is likely that there are more than one molecules or gene involved in cancer initiation and proliferation. These genes or molecules are the most fragile components of network and therefore the most vulnerable sites within the functional networks comprising the cancer-appropriate targets for therapeutic attacks. Since escaping apoptosis is one of the characteristics of cancer cells, apoptotic-targeted therapies can be used as on the treatment basis of cancer. Expression of epigenetically silenced CD95 (fas receptor) could be restored upon treatment with histone deacetylase inhibitors. TRAIL (TNF related apoptosis-inducing ligand) ligand/receptor system presents the most promising target for therapeutic intervention and clinical translation among the death receptors, as it can induce apoptosis in many tumor cell lines without affecting normal cells. There are a lot of approaches that have been used to counteract the effect of anti-apoptotic bcl-2 protein. Also inhibitors of apoptosis (IAP) can be exploited for cancer therapy like neural apoptosis inhibitory protein, cellular IAP1, melanoma-IAP.

The certitude that pathways involved in cancer progression like ras-ERK and AKT/mTOR dominate so many characteristics of cancer cells and its components that are so commonly mutated in variety of cancer which gives us hope that targeting them will give us successful results. Inhibitors targeting these pathways have been developed and many of them are in clinical trial now. A group of covalent small inhibitors have been developed and achieved encouraging treatment for ras-driven cancer. Several akt inhibitors are also designed like MK2206, AT7867, and KRX0401. Majority of akt inhibitors are based on binding mechanisms related to adenosine triphosphate (ATP). Further insights into these signaling pathways in response to anticancer drug treatment will likely have important implications for the development of molecular targeted therapies. Although the framework by which these signaling pathway and molecule interact with each other has been illustrated, the challenge faced is to know the precise molecular basis and their impacts on cancer therapies which remain remains unresolved.

References

1. Richard Sever, John S. Brugge, Signal transduction in cancer. Cold springer harbor perspectives in medicine; c2015. p. 21.
2. D'orta A, Del Buono A, De Monaco A. Nutritional manipulator: epigenetic effect in cancer. World Cancer Research Journal; c2014. p. 5.
3. Seyed Hussain Hassanpour, Mohanmadamin Dehghani. Review of cancer from perspective of molecular. Journal of cancer research and practice; c2017. p. 129.
4. Aide Negri, Valeria Naponelli. Molecular targets of epigalloccatechin- gallate (EGCG): a special focus on signal transduction and cancer. Nutrients; c2018. p. 10.
5. Julian Molina R, Alex Adjei A. The Ras/Raf/MAPK pathway. Pathway of the month; c2006. p. 9.
6. Scott W. Lowe and Athena W. Lin, Apoptosis in cancer. Carcinogenesis; c2000. p. 485.
7. Riccardo Fodde, Ron Smits, Hans Clevers. APC, signal transduction and genetic instability in colorectal cancer, Nature; c2001. p. 67.
8. Manuel Hidalgo, Eric Rowinsky K. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene; c2000. p. 19.
9. Geoffrey Clark J, Channing Der J. aberrant function of the Ras signal transduction pathway in human breast cancer. Springer link; c1995. p. 133.
10. Roberto Bianco, Davide Melisi, Fortunato Ciardiello, Giampaolo Tortora. Key cancer cell signal transduction pathways as therapeutic targets. European Journal of Cancer; c2009. p. 290.
11. Alexander Levitzki, Shoshana Klein. signal transduction therapy of cancer. Molecular Aspects of Medicine; c2010. p. 287.
12. Kaleigh Fernald, Manabu Kurokawa. Evading apoptosis in cancer. Trends in cell biology; c2013. p. 633.
13. Lin Zhang, Wei Zhou, Victor Velculescu E, Scott Kern E, Ralph Hruban H, Stanley Hamilton R, *et al.* gene expression profiles in normal and cancer cells. Science; c1997. p. 1272.
14. Nielsen LL, Maneval DC. Tumor suppressor gene therapy for cancer. Cancer gene therapy; c1998. p. 63.
15. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science; c1991. p. 53.
16. Mark Kieran W, Raghu Kalluri, Yoon-Jae Cho. The VEGF pathway in cancer and diseases: responses, resistance, and the path forward. Cold springer harbor perspective of medicine; c2012. p. 18.
17. Naoyo Nishia, Hirohisa Yano, Takashi Nishida, Toshiharu Kamura, Masamichi Kojiro. Angiogenesis in cancer. Vasc health risk management; ; c2006. p. 219.
18. Rak JW, St Croix BD, Kerbel RS. Consequences of angiogenesis for tumor progression, metastasis and cancer therapy. Anti-cancer drugs; c1995. p. 18.