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### New Treatments of Cancers using Gene Expression and Regulation

Even with the numerous advancements that have been made in the medical community, there are still diseases and disorders out there that have no known cause. This is exceedingly frightening for any patient with those mystery illnesses because without an identified cause, it is impossible to develop a cure. Cancer is a class of diseases that has always been under constant research. Even with multiple treatments, such as surgical incision of tumors, chemotherapy, radiation therapy, or adjuvant therapy, that can be utilized to eliminate cancer in patients, there are still loopholes and certain unfortunate circumstances that make cancer a devastating disease for any patient young or old. Fortunately, in recent years, as studies and innovations in the field of genomics have been more prevalent, this has encouraged many to research on the relationship between certain human cancers and their corresponding genes. As a result, scientists have discovered an important subfield of genetics that play a considerable role in the initiation and progression of cancers: gene expression and regulation.

Gene expression is when genes are expressed to produce functional RNA and proteins in the cell. This process occurs in two major stages called transcription and translation. In translation, the gene is copied to produce a complementary RNA molecule that consists of both exons and introns. As only the exons are needed to make proteins, the intron sequences are spliced out and a mature messenger RNA (mRNA) transcript with only exons leftover. During translation, ribosomes attach to the mRNA transcript and then move along it adding amino to the polypeptide chain (Twyman 3). The sequence of the mRNA is translated into a sequence of

amino acids in a protein where three nucleotides are required to generate one amino acid. Once the polypeptide chain is completed, it folds up to form the tertiary structure of the protein (Twyman 3).

Although there are the same quantity of most genes in every cell, genes in the human genome are not expressed in the same manner. This meaning that “the level at which a gene is expressed can vary from no expression to hundreds of mRNA copies per cell” (Berg, Tymoczko, Stryer 151). This difference in the pattern of when a gene is expressed is what distinguishes one cell type to another. For example, genes that encode actin and myosin are only expressed in muscle cells. This variation in how and when certain genes are expressed in particular cells results from the regulation of transcription and translation. Whether a gene is turned on or off is determined certain regulators that include RNA processing mechanisms, translation mechanisms, transcription factors, and epigenetic mechanisms. “Gene expression is therefore controlled by the availability and activity of different gene regulators” (Twyman 6). Moreover, regulators, such as transcription factors, are further monitored by other regulators. This ultimately means that regulation is necessary for normal gene and cell function. However, when there is a lack of or malfunction of a gene regulatory mechanism, a disruption of gene expression occurs, which eventually leads to the development of human cancers or tumors. For instance, it has been found that breast cancer susceptibility increases when a single copy of either the BRCA1 or BRCA2 genes is mutated. This is due to the fact that BRCA1 and BRCA2 genes assist in blocking cell cycle progression when there is DNA damage and in activating DNA repair to keep the sequence of the genome during cell division (Venkitaraman). Once these two genes are mutated, the check point control of DNA damage is eliminated, leading to incorrect gene expression, ultimately increasing the chances of obtaining breast cancer. However, by identifying BRCA1 and BRCA2

as genes that contribute to breast cancer susceptibility and their connection to other checkpoint genes involved in breast cancer, this allows scientists to focus and understand this signaling pathway and possibly develop treatments for these defective conditions.

Metastatic cancers are also caused by mistakes and disruption in the regulation of human gene expression. Metastasis occurs when cancer cells spread from the place where it first started to other parts of the body. Originally, metastatic cancers were believed to occur when a selective number of cells of a large primary tumor contained a set of genetic characteristics that permitted them to leave the original cancer site and grow in other organs (Sarasin, Kauffmann 49-50). This theory was eventually disregarded when it was found that most or all of the primary tumor cells had the same potential to show metastasis. This discovery then allowed scientists to conclude that metastatic cancers were associated to “cell adhesion, angiogenesis, cell cycle regulation, initiation of DNA synthesis, and DNA repair” (Sarasin, Kauffmann 49). In other words, metastasis is associated with gene expression and regulation. Colorectal cancer, for example, is the second leading cause of death in the US, not because it is incurable but because it has the ability to spread outside of the large intestine and attack other cells (Tsuji, Kawano, Dubois 1). In studying the effect of COX-2 expression in human colon cancer cells on the invasive potential of these cells, scientists discovered that the Caco-2 cells in large part caused the metastatic tendency of colorectal cancer. These Caco-2 cells, responsible for COX-2 expression, had acquired increased invasiveness in colorectal cancer patients when they “normally only express barely detectable levels of COX-2 protein in healthy patients (Tsuji, Kawano, Dubois 2). This change in the function of the Caco-2 cells was associated with the activation of metalloproteinase-2. When these abnormalities in regulation of this protein were detected, the increased invasiveness of the Caco-2 cells was reversed by a simple treatment with sulindac

sulfide, a COX inhibitor. Through this treatment, the likelihood of a metastatic spread of colorectal cancer cells decreases to a certain extent making the cancer less deadly.

Not only is metastasis caused by activation of certain proteins like in colorectal cancer, but it occurs when there is an overexpression of DNA repair genes. Sarasin and Kauffmann studied the biological pathways of human primary malignant melanoma, breast cancer, and bladder cancer and found that DNA repair pathways are overexpressed in tumors associated with the risk of metastasis. Their conclusion is logical in that the tumor cells that have tendencies to metastasize probably replicate and divide at faster rates. As a result, there needs to be increased DNA repair gene expression to control and stabilize the genome of primary tumor cells to give them enough stability to invade other organ sites (Sarasin, Kauffmann 53). This discovery concerning the overexpression of DNA repair pathways can be used to predict the correct clinical causes and outcomes of a cancer, which would be exceedingly beneficial to the patient. For instance, if the genes of the cells in a primary tumor indicated that metastasis is unlikely, then the patient will not have to undergo extensive and unnecessary treatments. This then allows more focus to be put towards patients with tumors that show greater likelihood of spreading to other organs. Additionally, by knowing which DNA repair pathways are overexpressed, inhibition of these pathways could lead to a possible treatment of commonly fatal metastatic cancers.

Methylation of DNA is another mechanism that that inhibits gene expression of a specific cell type. It does this by changing the expression of genes and by transmitting DNA methylation patterns through cell division, ultimately affecting epigenetics (the inheritance of information on the basis of gene expression levels) (Jones, Laird 1). DNA methylation is when the carbon 5 of cytosine is methylated by specific methyltransferases. The resulting methyl group of 5-methylcytosine sticks out into a major groove, where it then interrupts the binding of a protein

responsible for transcription. Moreover, DNA methylation affects a wide number of promoter sequences. These promoters are enriched with CpG islands that usually are not methylated. However, scientists have found an increase of methylation of these CpG islands in cancer cells (Jones, Liard 4). Because several tumor-suppressor genes contain CpG islands in their promoters, it makes sense that tumor-suppressor genes are probably silenced by methylation. This tumor-suppressor inactivation is a major cause of multiple cancers. In fact, a change in the methylation of a gene has been recognized to be the initial cause of 70 percent of cancers (Brutlag, lecture 11/12/08). As it has become evident that the state of methylation is a factor in the formation of tumor cancers, new techniques to scan the genome for methylation changes have been utilized to reveal new genes attacked by methylation in human cancers. The new genes, if correct, will permit scientists to get a clearer picture of how specific cancers begin and progress, providing valuable information that could be used to develop new and more effective treatments. Also, abnormal DNA methylation's ability to silence tumor-suppressor genes can be fixed clinically. It might be possible "to reverse the epigenetic changes and restore gene function to a cell" (Jones, Liard 4). An additional preventive treatment might be to inhibit abnormal DNA methylation, which would consequently slow down or discourage the development of cancers and tumors.

The main goal in analyzing the pathways of gene expression and regulation is to find more efficient and faster methods in diagnosing and treating human cancers. Not only have scientists used gene expression to find genes and cells that direct certain cancer genesis and used them as candidates for drug targets, but they've used the simple characteristic of gene expression to develop new treatments for certain cancers. It was stated before that how often a gene is expressed varies from one cell type to another. Scientists have used this significant variation in

cells and developed a powerful method that allows them to analyze the pattern and level expression of all genes in a cell or tissue (Berg 152). This method is the use of high-density arrays of oligonucleotides called DNA microarrays or DNA chips. These microarrays are constructed to detect variations in how often specific genes are expressed and have been used by scientists to better understand the cause of certain human cancers. For example, patients with promyelocytes have translocations that involve the rearrangement of the RARA gene, which is a feature of acute promyelocytic leukemia. The Pat Brown lab used microarrays to detect which genes were and were not expressed in patients with promyelocytes. They found that the patients with the disease lacked TGF-beta Cell signaling and needed insulin or retinoic acid receptors (Brutlag, lecture 11/17/08). Once this discovery was made, they gave the patients vitamin A, which then restored the signal. The receptors responded to the vitamin A the same way they did to the required TGF-beta cells. As time passed, the vitamin A, which was acting as a substitute, eventually caused the mutant stem cells to go away and normal cells started to form, eliminating the disease.

A molecular diagnostic company called Pathwork Diagnostics, Inc. has used the basic idea and function of microarrays or path chips to create an FDA-cleared gene expression test to assist in the diagnosis of the origin of uncertain tumors, especially in the case of metastatic cancers. This molecular diagnostic test “measure the expression pattern, comprising of more than 1,500 genes, in an uncertain tumor to compare it to expression patterns of a panel of 15 known tumor types, representing a total of 60 morphologies” (Pathwork Diagnostics). The use of gene expression and regulation by this company is not only innovative but allow for a quicker, more efficient, and less expensive way of finding where an unknown tumor comes from. Before Pathwork Diagnostics, physicians would have to use immunohistochemistry (IHC) and other

imaging tests to figure out the origin of metastatic tumors. It did not help that the results usually took weeks to come back, and even then, treatment of the patient was a trial-and-error type of diagnosis. This was both frustrating and extremely expensive for both the doctors, patients, and healthcare companies (Pathwork Diagnostics). However, with the gene expression test using microarrays, physicians are able to know the origin of their patients' tumors in a matter of seconds, which then allows them to the correct cancer-specific treatments.

Learning and researching more about gene expression and regulation of cancers will not guarantee better treatments, but it will give scientists a better look at how cancers initiate and progress. The key to finding a cure or treatment is to pinpoint the specific abnormality that caused the disease to develop. Unfortunately, this is easier said than done, but by focusing and analyzing the biological pathways of certain cancers, scientists will be able to see what regulation mechanism malfunction triggered a disease to develop. Gene expression and regulation might not be the key to the ultimate "cure to cancer", but it does signify that scientists and physicians are heading in the right direction.