Computational Biology:
Signals Depend On Several Factors

Based on the use of quantum biology, the following thread of documents was prepared for review and discussion with computational biologists to explain the next generation of computational biology and the critical nature of this discipline to all scientific research related to cellular physiology.

As outlined in the document affixed to this article, modeling of DNMT signaling has identified this family of signaling molecules to be byproducts of calcium – zinc – nitric oxide (IL-3) and catabolic activity on nitric oxide. Epigenetic research prior epigenetic modeling has, with near certainty, verified this configuration as being bioidentical to the troponins; C- T and T.

Quantum biology has provided irrefutable proof that the same signaling molecules can impact difference cells dependent of the receptors involved.

Given this fact, disruption of calcium - zinc with the amino acids being phenylalanine - tyrosine - tryptophan can lead to cancers as opposed to cardiac-related troponin if these constituents or the gasotransmitter is different. Complicating the use of computational biology is the fact that the same elements and amino acid configurations can have different outcomes if the gasotransmitter in the cytokine is H2S as opposed to NO due to downstream chirality mutations.

It should be noted that the failure of researchers to rely on computational biologists to account for elements and minerals as well as variations

between receptors for the same signaling molecule can continue to create mass confusion relative to the causes of chronic diseases.

Key epigenetic switch mechanism in gene regulation discovered

October 13, 2016 by Brian Wallheimer in Biology / Biotechnology

A Purdue University study pinpointed an epigenetic mechanism that is a key factor in how genes are switched on and off.

Both genetic and epigenetic mechanisms regulate human gene expression. External or environmental factors, such as carcinogens from tobacco smoking, disrupt normal epigenetic regulation. This leads to changes in gene expression, which results in the production of cancerous cells.

Humaira Gowher, a Purdue assistant professor of biochemistry, is interested in the mechanisms that control gene expression by directing epigenetic regulators such as DNA methylation to specific portions of a gene.

Gene expression is controlled by its genetic regulatory elements called promoters and enhancers. When cells need to express a specific gene, its enhancer element interacts with its promoter to stimulate the activation process. When a gene needs to be turned off or repressed, its specific enhancer is disengaged from the promoter.

DNA methylation refers to the addition of a methyl group to one of the bases of the DNA, cytosine, converting it into a methylcytosine. Presence of methylcytosine at the promoters and enhancers of genes signals the associated gene to be inactive.

DNA methylation is catalyzed by the enzymes called DNA methyltransferases or Dnmts.

Gowher and her team found that these Dnmts are important for releasing enhancers during gene repression and determined that a particular enzyme acts as a type of relay switch where the activity of one enzyme turns on the activity of the next, ultimately triggering an enzyme called Dnmt3a to methylate DNA in a specific location.

"The process we discovered provides a way for cells to control the activity of Dnmts at specific enhancers where DNA methylation must be deposited to ensure that genes are turned off when required," said Gowher, whose findings were published in the journal *Nucleic Acids Research*.

Gowher and her team studied this mechanism for a class of genes named pluripotency genes, which are expressed in stem cells. Stem cells replicate rapidly and stay in an undifferentiated state until they get an assignment and become a particular type of cell. During the process of cell differentiation, the pluripotent genes are turned off and DNA methylation occurs.

When external or environmental factors act on differentiated cells, DNA methylation can be disrupted, triggering a pluripotent state that leads to rapid proliferation of now damaged and cancerous cells.

"Understanding the way that cells regulate these mechanisms of repression may be able to help us understand what is being damaged and what we can watch for that can turn these genes back on," Gowher said.

Gowher said future research will involve looking further upstream in the process, particularly at the signals that can modulate the activity of these enzymes.

More information: Christopher J. Petell et al. An epigenetic switch regulates DNA methylation at a subset of pluripotency gene enhancers during embryonic stem cell differentiation, *Nucleic Acids Research* (2016). DOI: 10.1093/nar/gkw426

Provided by Purdue University

"Key epigenetic switch mechanism in gene regulation discovered" October 13, 2016 http://phys.org/news/2016-10-key-epigenetic-mechanism-gene.html

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MCFIP – Our findings relative to the impact of DNMTs are outlined in the documents affixed to this one; especially as they relate to breast cancer.

Scientists discover a genetic mechanism for cancer progression

September 11, 2015 by Jeannette Spalding in Medicine & Health / Genetics

Genetics researchers from Case Western Reserve School of Medicine have identified a novel long non-coding RNA (lncRNA), dubbed DACOR1, that has the potential to stymie the growth of tumor cells in the second-most deadly form of cancer in the U.S.—colorectal cancer.

The researchers found that this lncRNA is present in cells of healthy colons, but becomes suppressed in those carrying the disease. More importantly, this lncRNA interacts with a key enzyme known as DNMT1 that has important functions in all healthy cells of the body. Thus, the authors applied a name to this novel lncRNA—DACOR1, which stands for DNMT1-Associated Colon Cancer Repressed lncRNA-1.

The scientists' next challenge is to determine how to deliver DACOR1 to tumors where it may be able to slow, or even stop, the spread of malignant cells. The researchers' initial findings appeared in this month's edition of *Human Molecular Genetics*.

"We found that the metabolism of cancer cells slows when we put DACOR1 back in," said senior author Ahmad M. Khalil, PhD, an assistant professor of genetics and genome sciences. "If we could figure out a way to deliver DACOR1 to tumors, we could change the methylation patterns in cancer cells to either destroy or at least regress tumors."

DNA methylation affects the molecule's function, including gene expression. Whenever a biological process affects genetic expression, the potential exists to promote health or cause disease. To understand what factors decide which outcomes in humans, scientists first identify how different molecules are supposed to act—and, in turn, the elements that make their functions go awry.

Khalil, a member of the Case Comprehensive Cancer Center, began the journey to the team's discovery with a hypothesis—namely, that lncRNA molecules directly regulate the enzyme DNMT1 that adds methylation to DNA. This ongoing chemical modification of our DNA—a process known as DNA methylation—can help prevent cancer by maintaining a healthy level of gene expression, which in turn controls the extent of cell growth in the body.

During their research, investigators found that specific lncRNAs regulate DNA methylation in specific human genes. Researchers also sought clues on how lncRNAs affect normal tissue versus cancerous tissue and characterized one particular lncRNA, DACOR1, which is present in healthy colon tissue but missing from colon cancer tissue.

"Cancer cells do not want certain lncRNAs around because they instruct normal cells to grow at a specific rate," said Khalil, the study's senior author. "Our research, in part, explains how cancer cells change their DNA methylation pattern, and this change is a major mechanism where normal cells become cancer cells."

Methylation is a process where a methyl group is added into critical points along our DNA. In cancer, many regions of the DNA are not methylated, leading to untimely gene expression, and unwanted cell reproduction. In some instances, genes that trigger cancer, known as oncogenes, spring into action.

DNA methylation occurs through enzymes known as DNA methyltransferases (DNMTs), and there are only three of them in the human genome: DNMT1, DNMT3A and DNMT3B. DNMT1 is the most important one because of its activity in all cell types, so investigators focused on how a subset of lncRNAs might interact with DNMT1. Khalil's hypothesis was that a subset of lncRNAs interacts with DNMT1 and that cancer cells alter the expression of these specific lncRNAs to change the location of where the DNMT1 enzyme triggers methylation along the DNA.

In their research, Khalil's team first isolated 148 lncRNAs from 8,300 known lncRNAs. These 148 lncRNAs are associated with DNMT1 in colon cells. To test their hypothesis in depth, the researchers focused their study on a key lncRNA that becomes suppressed in colon cancer—DACOR1.

Then investigators obtained normal and cancerous colon tissue from public datasets and compared how DACOR1 acted within each. They found that DACOR1 is active in normal colon tissue but repressed in colon cancer tissue. Similar results were found in studying 21 colon cancer cell lines from the Markowitz Laboratory of Genetic Colon Cancer Research at Case Western Reserve—DACOR1 is suppressed in colon cancer.

Investigators also pondered whether returning DACOR1, and therefore, DNA methylation, to colon cancer cells would slow their growth. The answer was yes. In two colon cancer cell lines, the DNA methylation pattern changed when the lncRNA DACOR1 was injected into the cells. Additionally, investigators placed DACOR1 into

cancer cells to track where this specific lncRNA went. DACOR1 returned to those cancer-suppressing regions of the DNA where it had been missing in the cancer cells.

"These experiments showed us that DNMT1-associated lncRNAs are regulating DNA methylation and subsequent gene expression, which controls cell growth," Khalil said.

Next steps for Khalil's team will be more research into DACOR1 and the effects of other lncRNAs believed to suppress colon cancer. Perhaps defects in a number of lncRNAs work in synergistic unison to drive the onset of colon cancer. Eventually, the potential is there to develop a therapy that would reactivate key lncRNAs in their job of maintaining normal, controlled cell growth and prevent unbridled cell growth leading to cancer. If nothing else, the lack of colon cancer-repressing genes could serve as an early-detection biomarker for colon cancer.

"Clearly, cancer cells have a way of changing gene expression in a way that is advantageous to them, but how they do that, we don't entirely understand yet," Khalil said. "We do know now that cancer changes the expression of key cancer-suppressing lncRNAs in a way that is advantageous to cancer cell proliferation."

Provided by Case Western Reserve University

"Scientists discover a genetic mechanism for cancer progression" September 11, 2015 http://medicalxpress.com/news/2015-09-scientists-genetic-mechanism-cancer.html

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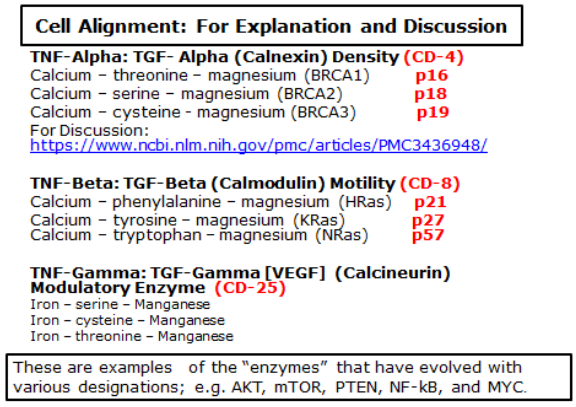
MCFIP – Modeling of the epigenetic markers referenced in the article produced the following results:

- DNMT1 is a member of a large family of zinc finger-calcium binding signaling molecules; i.e. making it likely to be a byproduct of IL-3
- This signaling molecule is part of a trefoil of DNMT1 -3 that have the amino acid constituents of phenylalanine, tyrosine and tryptophan. Accordingly, with near certainty, they are bioidentical to Troponin C – I and T

- ISL1 is a member of the trefoil consisting of ISL1 – 3. Our modeling indicates they are bioidentical to the signaling molecules for motility of spatial alignment; i.e. 14-3-3/TJPs. The amino acid constituents of these IL-32 byproducts are phenylalanine, tyrosine and tryptophan. Refer to the following for discussion relative to IL-3 calcium - zinc v IL-32 calcium - magnesium.



Reference to ISL1 prompted us to check our database to determine if previous modeling had identified the signaling molecule. The results of that process are included in the document affixed to this article; i.e. relative to congenital heart defects.

Summary

The results of our modeling effort have provided a verifiable foundation from which spatial alignment of breast cells can be linked to the "most common types of breast cancer" (as quoted in the article).

It should be noted that links between DNMT1 and ISL1 are noteworthy because both have phenylalanine as the amino acid constituents. This observation supports our hypotheses that pathways involving common amino acid constituents are likely to be a common factor in many cancers.

Gene found that is essential to maintaining breast and cancer stem cells

May 11th, 2015 in Genetics /

The gene and hormone soup that enables women to breastfeed their newborns also can be a recipe for breast cancer, particularly when the first pregnancy is after age 30.

Researchers have now found that the gene DNMT1 is essential to maintaining breast, or mammary, stem cells, that enable normal rapid growth of the breasts during pregnancy, as well as the cancer stem cells that may enable breast cancer. They've learned that the DNMT1 gene also is highly expressed in the most common types of breast cancer.

Conversely, ISL1 gene, a tumor suppressor and natural control mechanism for stem cells, is nearly silent in the breasts during pregnancy as well as cancer, said Dr. Muthusamy Thangaraju, biochemist at the Medical College of Georgia at Georgia Regents University and corresponding author of the study in the journal *Nature Communications*.

"DNMT1 directly regulates ISL1," Thangaraju said. "If the DNMT1 expression is high, this ISL1 gene is low." They first made the connection when they knocked out DNMT1 in a mouse and noted the increase in ISL1. Then they got busy looking at what happened in human breast cancer cells. They found ISL1 is silent in most human breast cancers and that restoring higher levels to the human breast cancer cells dramatically reduces the stem cell populations and the resulting cell growth and spread that are hallmarks of cancer.

When they eliminated the DNMT1 gene in a breast-cancer mouse model, "The breast won't develop as well," Thangaraju said, but neither would about 80 percent of breast tumors. The deletion even impacted super-aggressive, triple-negative breast cancer.

The findings point toward new therapeutic targets for breast cancer and potentially using blood levels of ISL1 as a way to diagnose early breast cancer, the researchers report. In fact, they've found that the anti-seizure medication valproic acid, already used in combination with chemotherapy to treat breast cancer, appears to increase ISL1 expression, which may help explain why the drug works for these patients, he said. The scientists are screening other small molecules that might work as well or better.

Mammary stem cells help maintain the breasts during puberty as well as pregnancy, both periods of dynamic breast cell growth. During pregnancy, breasts may generate 300 times more cells as they prepare for milk production. This mass production may also include tumor cells, a mutation that seems to increase with age, Thangaraju said. When the fetus is lost before term, immature cells that

were destined to become breast cells, can more easily become cancer, said Rajneesh Pathania, a GRU graduate student and the study's first author.

DNMT1 is essential for maintaining a variety of stem cell types, such as hematopoietic stem cells, which produce all types of blood cells. But, its role in regulating the stem cells that make breast tissue and enable breast cancer has not been studied, the scientists write.

While the exact reasons remain unclear, there is an increased risk of breast cancer if the first pregnancy occurs after age 30 as well as in women who lose their baby during pregnancy or have an abortion. Women who never have children also are at increased risk, while multiple term pregnancies further decrease the risk, according to the American Cancer Society.

Theories include that the hormone-induced maturation of breast cells that occurs during pregnancy may increase the potential for breast cancer cells to be made as well. Also, most breast cancers thrive on estrogen and progesterone, which are both highly expressed during pregnancy and also help fuel stem cell growth.

During pregnancy, stem cells also make more of themselves so their population increases about five times. DNMT1 levels experience a similar increase.

In five different types of human breast cancer, researchers found high levels of DNMT1 and ISL1 turned off. Even in a laboratory dish, when they put the ISL1 gene back, human breast cancer cells and stem cell activity were much reduced, Thangaraju said.
Provided by Medical College of Georgia

"Gene found that is essential to maintaining breast and cancer stem cells." May 11th, 2015. http://medicalxpress.com/news/2015-05-gene-essential-breast-cancer-stem.html

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MCFIP – There are critical points to consider with regard to this research; i.e. change references from proteins to signaling molecules and transcription factor to cell-surface signaling molecule.

Our modeling of the 14-3-3 protein (signaling molecule) indicates it is, with near certainty, likely to be the same as tight junction proteins or desmosomes; all of which signal for the motility of cells.

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Web address:
http://www.sciencedaily.com/releases/2012/07/120712131601.htm

Finished Heart Switches Stem Cells Off

=ScienceDaily (July 12, 2012) — Transcription factor Ajuba regulates stem cell activity in the heart during embryonic development. It is not unusual for babies to be born with congenital heart defects. This is because the development of the heart in the embryo is a process which is not only extremely complex, but also error-prone. Scientists from the Max Planck Institute for Heart and Lung Research in Bad Nauheim have now identified a key molecule that plays a central role in regulating the function of stem cells in the heart. As a result, not only could congenital heart defects be avoided in future, but new ways of stimulating the regeneration of damaged hearts in adults may be opened up.

It's a long road from a cluster of cells to a finished heart. Cell division transforms what starts out as a collection of only a few cardiac stem cells into an ever-larger structure from which the various parts of the heart, such as ventricles, atria, valves and coronary vessels, develop. This involves the stem and precursor cells undergoing a complex process which, in addition to tightly regulated cell division, also includes cell migration, differentiation and specialisation. Once the heart is complete, the stem cells are finally switched off. MCFIP – Our modeling of the TPJ signaling molecules indicates they regulate cellular adhesion (motility) in conjunction with their cell-surface counterparts (condensation). The Condensation signaling molecules are known as the family of SynCAM 1- 3. Numerous studies identify these signaling molecules with the development of the heart.

If either "family" is disrupted, outcomes would like be a congenital defect. Determining which part of the heart being impacted will require considerable research. Regardless, if our modeling is correct, theranostic testing and early intervention through therapeutic strategies may prevent the negative outcomes.

Scientists from the Max Planck Institute for Heart and Lung Research in Bad Nauheim have now discovered how major parts of this development process are regulated. Their search initially focused on finding binding partners for transcription factor Isl1. Isl1 is characteristic of a specific group of cardiac stem cells which are consequently also known as $Isl1^{+}$ cells. During their search, the researchers came across Ajuba, a transcription factor from the group of LIM proteins. "We then took a closer a look at the interaction between these two molecules and came to the conclusion that Ajuba must be an important switch," says Gergana Dobreva, head of the "Origin of Cardiac Cell Lineages" Research Group at the Bad Nauheim-based Max Planck Institute. MCFIP – Mention of ISL1 in conjunction with Ajuba is significant because, when subjected to our modeling process, this signaling molecule was identified as being part of a trefoil consisting of ISL1 - 3 that is bioidentical to the 14-3-3/TJPs. In other words, these factors support the likelihood that spatial alignment is responsible for the congenital defects discussed in this article.

Using an animal model, the scientists then investigated the effects of a defective switch on cardiac development. Embryonic development can be investigated particularly effectively in the zebrafish. The Bad Nauheim-based researchers therefore produced a genetically modified fish that lacked a functioning Ajuba protein. Cardiac development in these fishes was in fact severely disrupted. In addition to deformation of the heart, caused by twisting of the cardiac axis, what particularly struck the researchers was a difference in size in comparison with control animals. "In almost all the investigated fish we observed a dramatic enlargement of the heart. If Ajuba is absent, there is clearly no other switch that finally silences the Isl1-controlled part of cardiac development," says Dobreva.

Further investigations revealed that the enlargement of the heart is in fact attributable to a greatly increased number of cardiac muscle cells. The reason for this was in turn that the number of $Isl1^{+}$ cells, i.e. the cardiac muscle precursor cells, was distinctly raised right from an early phase of development. Ajuba is a decisive factor in controlling stem cell activity: it binds to Isl1 molecules, thus blocking their stimulant effect.

The results from the study could have potential future applications. "Once we understand how cardiac development is regulated, we will also be more familiar with the causes of congenital heart defects and will consequently be able to consider therapeutic approaches," comments Dobreva. Damaged adult hearts can also be repaired in this way: "One possibility would be to optimise the production of replacement cells from embryonic or artificially produced stem cells in the laboratory. Silencing Ajuba in these cells might enhance their development into functional cardiac muscle cells. Sufficient replacement cells for treating patients could be cultured in this way." Another possibility is to stimulate stem cell activity by silencing Ajuba in the damaged heart and so cause the heart to regenerate itself. Further studies are now set to investigate how feasible this might be.