The Genetics of Granulosa Cell Tumour

An Unofficial Guide for the Scientifically Illiterate

Based on the research of Dr. Paul A. Cohen and Ms. Roseanne

Rosario and in partnership with:

 The University of Auckland Faculty of Medical and Health Sciences

 The Granulosa Cell Tumour Foundation of New Zealand

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About the Author

Haley is a sophomore Economics and Pre-Medical undergraduate student at Washington & Jefferson College in Washington, Pennsylvania. Since being diagnosed with granulosa cell tumour in 2009, she has been active in raising awareness in her local community and passionate about ovarian cancer research. Haley currently lives outside Pittsburgh, Pennsylvania with her family and wishes to pursue a career in either medicine or health economics after graduation.

Acknowledgements

Dedication

I now believe that it is possible for people other than the science-inclined to understand current cancer research. I am living proof. However, based on what is published, the rest of the world does not always seem to agree. Have you ever tried to read a 200-page thesis on the genetics of cancer? I do not recommend it unless you have a medical dictionary, a Ph.D., and a lot of time. Read this instead. Trust me.

My mission during my five-week internship with the Granulosa Cell Tumour Foundation of New Zealand and the University of Auckland Faculty of Medical and Health Sciences was to better understand the complex genetics research being conducted on a rare form of ovarian cancer called granulosa cell tumour (GCT) and translate it into a paper which both academics and regular people, like you and me, could comprehend. No posh vocabulary. No cryptic explanations. Just the facts, plain and simple. This is what I have devised.

To my fellow survivors I say: this final product is ultimately for you. I hope that it helps you better understand the science of your disease. Sometimes, we are under the impression that since we are rarities, no one cares about curing GCT, and when abstract discoveries are published, they do not seem practical or sensible because we cannot understand their importance. I assure you that, at the very least, there is a whole team of people in Auckland who are working hard for you, and the fascinating, albeit occasionally confusing, research being done there is 100% worthwhile.

If you do not believe me, take a look for yourself.

In memoriam

Sherry Snyder, Janice Bublitz, Diane Seibert, Janet Torina, and Sladjana Crosley

who battled GCT until the very end.

Introduction to Female Reproductive Function and Ovarian Cancer

Granulosa Cell Function

Granulosa cells are the main cell type associated with aiding the oocyte (also known as the egg) in the ovarian follicle. These cells continuously divide to surround the oocyte, forming a structure that protects and nurtures the oocyte throughout its development.   Granulosa cells also secrete oestrogen, a prominent female hormone, as well as other important growth factors to allow the follicles to become larger. Once the oocyte has reached full maturity, it leaves ovary. This process is called ovulation.

As the oocyte leaves the ovary alone, the granulosa cells of the follicle remain in the ovary and undergo a process called apoptosis. This basically means that they are now programmed to die, allowing for the aforementioned cycle to repeat itself. However, a deregulated version of this process where granulosa cells exponentially divide but never die may be the basis GCT.

The Origins and Nature of Ovarian Cancer and GCT

There are three main types of ovarian cancer: The overwhelming majority of cases (85%) originate from epithelial ovarian cells found on the outermost surface of the ovary. The second type of ovarian cancer originates from oocytes and leads to germ cell tumours. However, we are interested in cancers arising from the last category of ovarian cells – sex cord or stromal cells, which are cells that support the development of oocytes.   GCTs are the predominant malignancy in this subgroup of ovarian cancer (Figure 1A).
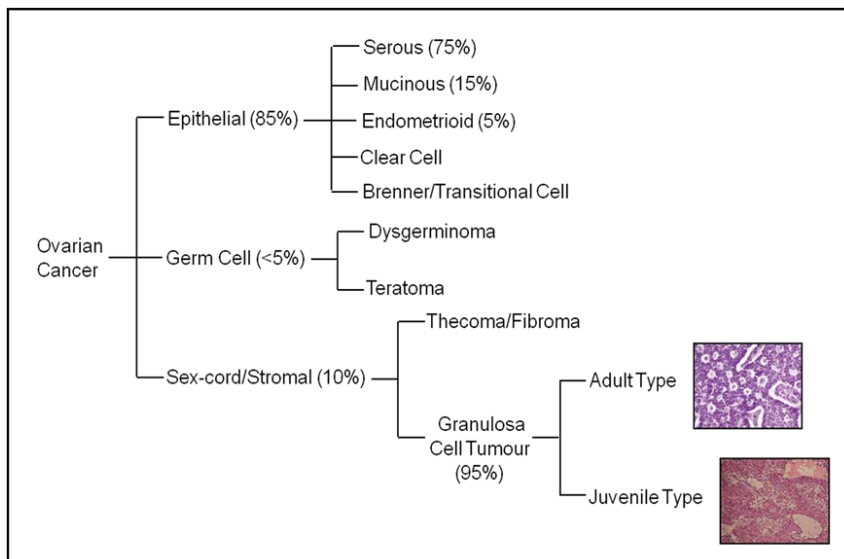

Figure 1A shows a flowchart of the categories of different ovarian cancers and their incidences.

GCTs form in follicular granulosa cells, which are granulosa cells furiously dividing and gathering around an oocyte during all stages of follicular development. Most commonly, GCTs occur in post-menopausal women traditionally in their mid-fifties, though a small yet non-negligible amount of GCTs pop up in prepubescent girls. This age gap in patients encouraged scientists to compare the tumours of the younger and older patients. They discovered that there were cellular differences between the tumours and split the classification of GCTs into two groups: adult and juvenile. However, age does not necessarily indicate what kind of GCT a woman has; a young girl may harbour an adult type malignancy while an older woman may present with a juvenile tumour. Adult and juvenile GCTs indeed have different pathological qualities and, when grown in culture, have different appearances (Figure 1B).
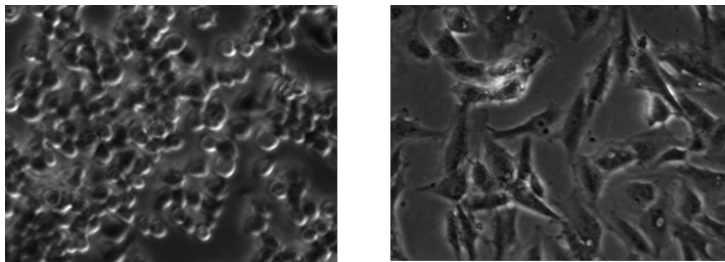

Figure 1B: The left picture shows a slice of juvenile tumour grown in culture and enhanced with a microscope. On the right is an adult tumour also grown in culture. There are obvious differences between their cellular appearances.

Although GCTs are comprised of different cells than epithelial tumours, the tumours are often treated the same simply due to the fact that GCTs are rare and there is little information available regarding their optimum treatment strategies. GCT statistics may be bleak because of blanket treatments, and there is a thought that GCT's need to have a GCT-specific treatment regime. Unlike epithelial ovarian cancer, GCTs tend to recur very late, sometimes 30 or 40 years after an initial diagnosis, which means that life-long vigilance is necessary to quickly catch a likely second bout of disease. The probability of recurrence in GCT patients is rather high (35%), and 80% of those with recurrent disease will die from it. Short term survival for women with GCT is good (anywhere from 60% to 90%), but the 25-year survival rate is less promising (40% to 60%). Bear in mind, however, that a large proportion of GCT patients are post-menopausal and may die of natural causes or other health problems within 25 years of their diagnosis, making longer term survival rates look worse than they really are. For instance, a 70-year old woman, with or without cancer, is unlikely to live another 25 years. That should be of some comfort, but the rates are still alarming.

The underlying cause of GCT remains unknown, but one theory suggests that GCTs form because consistently high Follicle Stimulating Hormone (FSH) levels trigger abnormal cell growth. FSH is a

hormone that is released from the pituitary and acts on the granulosa cells to encourage cell growth or division. To understand how this FSH hypothesis makes sense, we must review what happens when the female body undergoing menopause.. Throughout their lifetime, under the influence of FSH, women ovulate every month. Oocytes of better quality are often ovulated earlier in life, so by the time a woman reaches menopause, only follicles of poorer quality are left. To ovulate these poorer quality follicles, a lot of hormonal stimulation by FSH is required. The well known 'Popcorn Hypothesis' describes this phenomenon well. Think about popping popcorn the old-fashion way over a flame in a pan: initially, the kernels are eager to pop, and they continue to do so over even heat. As the tin overflows with popcorn, however, those last few little kernels at the bottom are stubborn and extra heat is required to make them pop. A post-menopausal woman's follicles which really don't want to ovulate anymore are like stubborn kernels– they need more hormonal stimulation (via FSH) to do their job (like turning up the heat to get the last kernels to pop), and the already high FSH levels get even higher (Figure 2). A younger woman though has enthusiastic follicles, and not as much FSH is needed to create the desired effect. The proposed theory on GCT formation is plausible considering that a large percentage of GCT patients are postmenopausal women with elevated FSH levels; however, it fails to provide an explanation for GCT in prepubescent girls and women of reproductive age.

On another note, granulosa cells in the follicle produce another hormone called Inhibin. The more follicles a woman has, the higher her level of Inhibin. Inhibin is a hormone that can suppress the secretion of FSH from the pituitary, meaning that the more Inhibin there is, the less FSH there is. So, post-menopausal women, who have fewer follicles, have less Inhibin and more FSH.
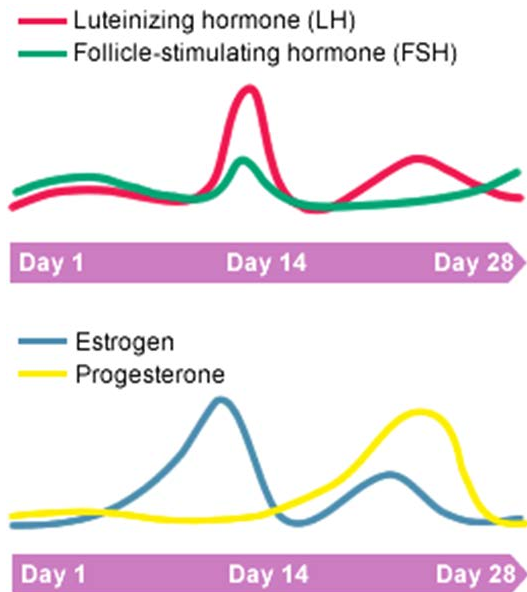
Figure 2: Assuming a 28-day menstrual cycle with ovulation happening at Day 14 and menstruation at Day 1, this graph shows the concentrations of hormones in a female based on the time in her cycle. As stated, FSH peaks at ovulation and then declines, as does LH.

Genetics also drives the creation of a tumour. When genetic material is accidentally mistranslated in the process of DNA replication and cell division, mutations occur, and they affect how those cells operate. In terms of cancer, a genetic mutation exponentially increases the rate of cell growth or prevents them from dying. So, obviously, there are differences in the genes of a normal granulosa cell and a cancerous granulosa cell in a GCT. Understanding these genetic differences can enable scientists to design potential therapies specific to combatting GCT. These genes and their associated DNA mutations are what scientists in Auckland are researching. Do not fret if you have not the slightest clue about genetics; I am going to explain all what it means in a minute.

Introduction to Cellular Biology and Genetics

The Cell

Human beings are just large, intelligent masses of cells. Large, isolated compilations of cells form organs, tissues, muscles, and other parts of our body. A cell, though small, is a very complex structure with multiple working sections (called organelles) each with a different job to keep the cell alive. The most important organelle is called the nucleus which acts as a cell's brain (Figure 3). It holds all of the genetic information a person has. Each cell, regardless of its position in the body, contains a complete copy of the human genome; however, cells only use the information necessary for them depending on their function, and the rest are dormant. For example, an ovarian cell does not need to utilize the piece of genetic code that determines what colour your eyes are but has it anyway. Obviously, that is crucial for cells in your eye to access. So, your eyes are said to express the eye-colour gene.
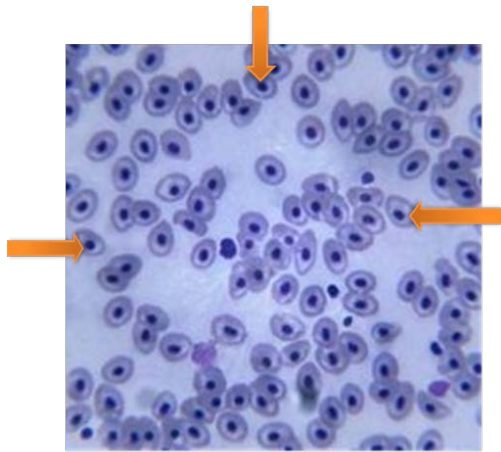
Figure 3: Orange arrows indicate nuclei, the dark circles within each cell, in a sample

DNA is the 'genetic code' that makes up genes and is held in thin, tight cylindrical coils in the nucleus called chromosomes. Every person has 22 pairs of chromosomes – within the pairs, one half is inherited from his or her mother and the other is from his or her father – and two sex chromosomes which determine if the individual is male or female. A woman has two X chromosomes, and a gentleman carries an X and a Y (Figure 4). Remember that every cell carries all 22 pairs of chromosomes and both sex chromosomes, but not every cell uses them all in daily function.
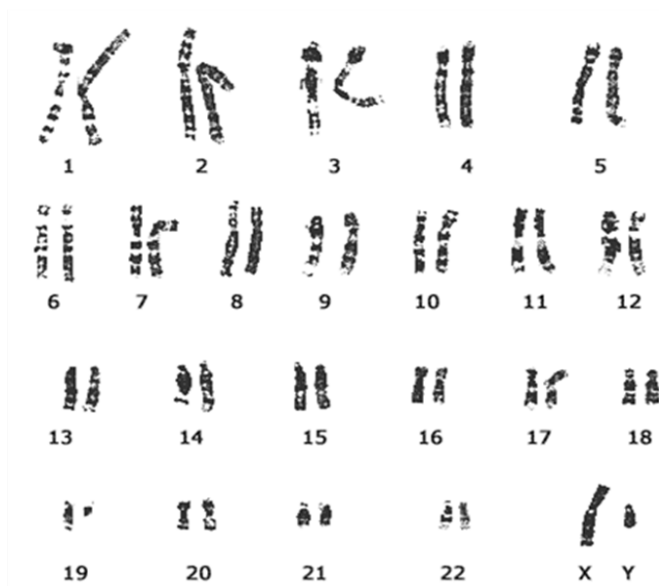
Figure 4 shows a complete set of human chromosomes in a male
(notice the X and Y instead of X and X)

Genes, which determine physical features, anatomical make-up, and susceptibility to disease lay in and are spread among the chromosomes. For instance, one of the genes that control colour-blindness is on the X chromosome while chromosome 15 holds roughly one-third of the genes that decide a person's skin tone, and some of the genes that determine a person's hair colour are located on Chromosome 19. However, not everyone expresses the same version of those genes; a blonde does not express the same version of the hair colour genes as does a brunette, for instance.
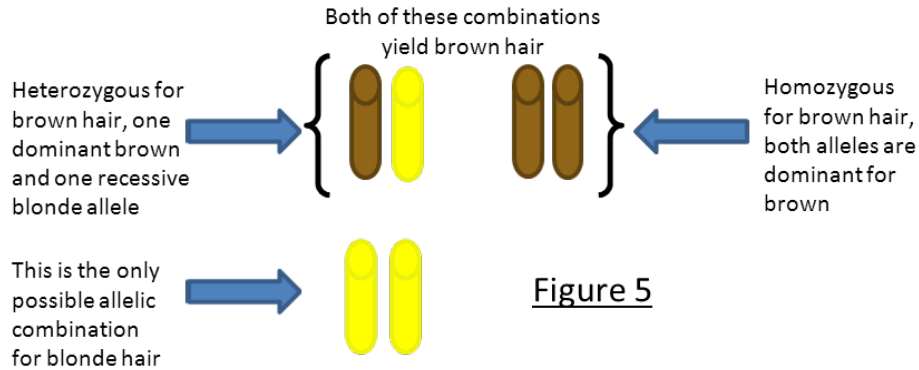
If we analyse a person's hair colour by studying genes, we must look at the chromosomes individually. Remember, we said earlier that a mother and a father each contribute a half of their child's chromosomes. Assuming there are four colours of hair (black, brown, red, and blonde), hair colour is based on dominant and recessive allele traits. There are two genes involved in hair colour: one affects darkness of hair, and the other controls red tinting. Take, for example, a woman named Debbie with blonde hair which is a recessive trait: she has two recessive blonde alleles, and there is no other combination of alleles that yields her hair colour. Her husband Frank has the dominant brown hair colour, but he has more possible allelic combinations. Dominant traits, like brown hair, only need one dominant allele to visibly express themselves; however, brown haired people can also carry two copies of the dominant allele (Figure 5). If Frank has only one dominant allele, his chromosome is heterozygous, and if he has two, it is homozygous. For our story's sake, say Frank is heterozygous for brown hair, meaning he carries a dominant brown hair allele and a recessive blonde hair allele.

Both of these combinations
yield brown hair

Heterozygous for
brown hair, one
dominant brown
and one recessive
blonde allele

Homozygous
for brown hair,
both alleles are
dominant for
brown

This is the only
possible allelic
combination
for blonde hair

Figure 5

Debbie and Frank decide to have a child – what colour hair will he or she have? Scientists use a Punnett square to figure this out (Figure 6). Debbie can only contribute a blonde allele to her child, so the fate of their offspring's hair colour rests in Frank's sperm. If Frank adds a brown hair allele to his wife's blonde one, their child will have brown hair (the brown allele "wins" dominance), and their child will be heterozygous for brown hair, like Frank. However, if Frank gives his recessive blonde allele, it will combine with Debbie's blonde allele to create a blonde child. If Frank were homozygous for brown hair though, there would be no possibility for a blonde child.

Debbie

	b	b
B	Bb	Bb
b	bb	bb

Frank
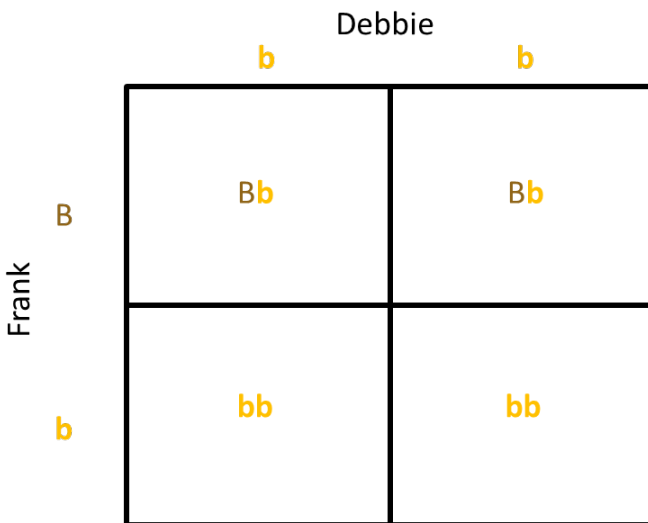
Figure 6 shows a Punnett square. B stands for the dominant brown hair allele and b stands for recessive blonde hair. All possible pairings of their alleles, shown in this diagram, are possible hair colors for their child. Bb is heterozygous brown hair and bb is blonde. There is a 50% chance that the child will have brown hair and a 50% chance that he or she will be blonde.

So, to recap: Your body is made up of organs which are just large communities of similar cell types, all of which have a nucleus that contains chromosomes made up of DNA.

DNA and the Study of Genetics

Your genes are your DNA. Depending on how your DNA is organized, your genes "say" different things. Going back to the colour-blindness example from paragraphs back, my DNA is put together in such a way that the colour-blindness gene in my X-chromosome appears in one particular version and therefore tells my eyes to distinguish varying colours. However, a colour-blind person has a different version of DNA in his X-chromosome that activates it. We both physically possess the gene that determines colour-blindness, but the differences in our DNA change the ability of our eyes to determine colours. These small variations in DNA are called variants, and if they cause disease they are called mutations. Most variants are not lethal to humans (like the colour-blindness example). In fact, everyone in some capacity has millions of variants in his or her DNA, but when a certain mutation occurs in a particular gene that causes something to go haywire, catastrophic diseases like cancer can sometimes occur. Fortunately these types of mutations are rare, and cancer does not occur in everyone.

Here is a good metaphor for understanding the difference between benign and potentially malignant DNA variants: Consider the sentence "the cat sits on the mat." Disregarding the fact that it is cheesy and reminiscent of Dr. Seuss literature, the sentence makes sense. The phrase is representative of a piece of DNA that has absolutely no change. Now, if the sentence is changed to "the cat sits around the mat," it makes less immediate sense, but maybe you can still draw some type of mental image for this scenario. This statement acts as a benign variance; the word "around" does not properly convey the image from before, but it is workable. The sentence "the cat sits between the mat" symbolizes a malignant mutation. It makes no sense at all, and if the body cannot understand a variant strand of DNA, disorders and diseases occur.

While there are discrepancies between harmless and dangerous mutations of DNA, there are also differences between inheritable and non-inheritable ones. Inheritable mutations are called germline mutations because they only occur in germ cells. In a woman's case, a germ cell (also called an oocyte or egg in the introduction) matures in a follicle, leaves the ovary, and after contact with sperm eventually becomes an embryo. If at any stage the germ cell experiences a mutation in its DNA, it will be passed to the child.

Somatic mutations are acquired mutations and cannot be transferred to future generations because they do not happen in germ cells; they can occur in somatic cells such as liver cells, skin cells, kidney cells, and anywhere else in your body. A person who fancies sun tanning, for example, may incur a change in his or her skin cells, but this mutation will not affect anyone's health but her own; that is, the damaged cannot be passed on. Depending on the severity of the mutation, she could develop

melanoma, but her children do not have a higher risk of skin cancer unless they adopt their mother's sun tanning habits. These observations directly relate to determining hereditary cancer risk factors – because GCTs originate in granulosa cells and not in germ cells, scientists do not consider GCT an inherited disease.

The structure of DNA, discovered in 1953 by James Watson and Francis Crick, is rather famous – the double helix (Figure 7A and 7B). It looks like a twisted ladder. Each "rung" of the ladder is a pairing of two of the four bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Adenine and thymine are always together, as are guanine and cytosine. The "sides" of the ladder are made of sugars and phosphates (do not worry if you do not know what these are; they are not mentioned after this). The bases are the most important component of DNA, as different combinations of the letters A, G, C, and T, which represent varying orders of the bases on a single strand of DNA, are what determine a unique human build. So, a small mutation in DNA which affects a tumour suppressor gene could turn it off, leading to uncontrolled tumour growth.
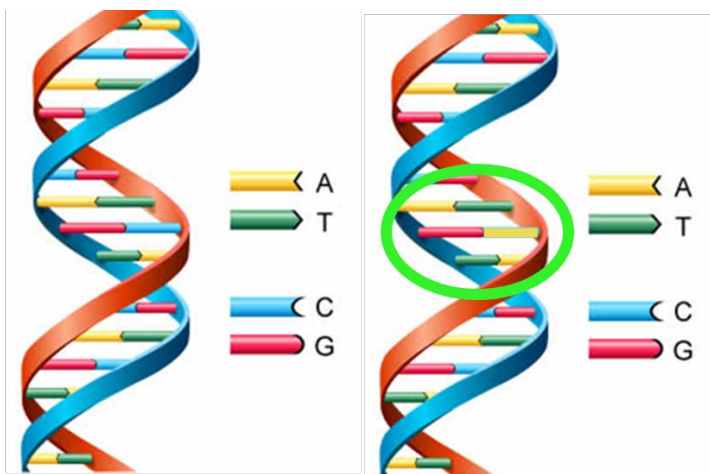
Figure 7A   Figure 7B

Figure 7A shows a double helix with no
mutation – all bases that are paired up
are complementary. Figure 7B, however,
shows a mutation in the green circle.
Adenine and guanine are paired together,
and they are not supposed to be.

Determining the differences between the expressions and mutations of tumour suppressor genes in GCTs and in normal samples will help to develop more effective treatments and futuristic genetic therapies specifically designed for GCT. This is precisely the researchers' goal at the Granulosa Cell Tumour Foundation New Zealand and the University of Auckland.

Recent and Current Research on Granulosa Cell Tumour

Overview

While many qualified scientists aid them in their studies, the main GCT genetics research experts at the University of Auckland and the Granulosa Cell Tumour Foundation New Zealand are Dr. Paul Cohen and Ms. Roseanne Rosario under the supervision of Dr. Andrew Shelling. This section has been paraphrased directly from their hundreds of pages of post-graduate theses.

The topics they focus on are FOXL2 and Oestrogen receptor beta. Both are believed to be abnormally expressed in GCTs for different reasons; knocking down and /or increasing the activity of the gene in malignant and normal cells may lead to a better understanding of their role in cancer development and possible new genetic therapies to fight and maybe even cure GCT.

FOXL2

Every cell in your body physically contains every gene – remember we said that in the introduction – but not every cell is required to activate all the genes to do its job. The gene FOXL2 is only essential for cells in the ovary, pituitary gland, and eyelid for a person to develop normally. So, therefore, it is unlikely that FOXL2 is the source of any mutations outside of the ovary, pituitary gland, and eyelid. The FOXL2 gene, located on chromosome 3, is thought to play an important role as a tumour suppressor (Figure 8).

Figure 8 shows a red line where the FOXL2 gene is located on chromosome 3
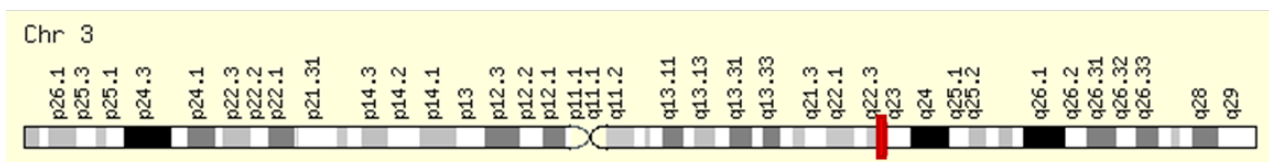
Most genes create proteins which are useful and necessary to the cell for different reasons. There are many different types of proteins, and each serves a unique purpose: some are hormones, some are enzymes, and others form structural proteins to make hair, skin, and nails. However, the FOXL2 gene creates a type of protein called a transcription factor.

Transcription factors help regulate the expression of other genes by recognising particular sequences of DNA in a place on the gene called a binding site. There are many types of transcription factor, but each individual gene's binding site is only compatible with specified transcription factors. For example, imagine you have a tool box with screwdrivers, each with a different head – one has a Philips head, another is a flathead, one is a hex, and so on. When confronted with a loose screw inside a child's toy, you choose the screwdriver that fits into the grooves of the screw you are attempting to fix; if the

screw is compatible with a Philips head, you reach for a Philips head screwdriver, and a flathead one would not work. This analogy can be applied to the process of transcription factors choosing what genes to help (Figure 9). If a gene has a FOXL2 binding site, then the FOXL2 transcription factor would help it. A transcription factor derived from another gene will not try to aid a gene compatible with FOXL2.

FOXL2 transcription factor: it's shape allows it to recognize certain patterns of DNA on Gene 1 but not on Gene 2

This other random transcription factor's different shape allows it to recognize other certain patterns of DNA. It can help Gene 2 but not Gene 1.

Gene 1

Gene 2

Figure 9 shows a chromosome with Gene 1 and Gene 2 on it. Each gene is only compatible with transcription factors that recognize its binding site's DNA code. Transcription factors identify different binding sites due to their unique shapes.
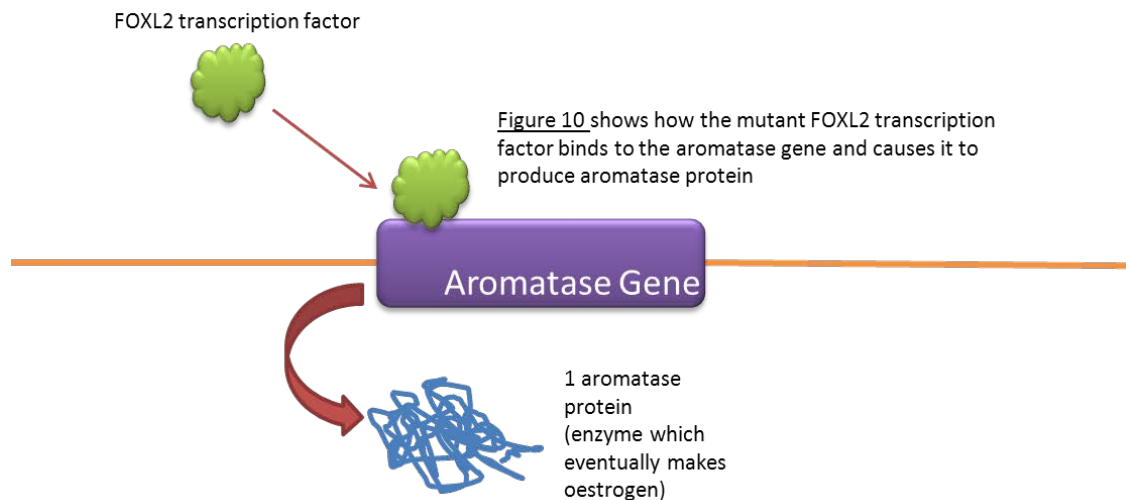
So, the FOXL2 gene mass-produces the FOXL2 transcription factor which is only able to bind to and help create proteins in genes with FOXL2 binding sites. Part of a research project in Auckland involves determining which genes require FOXL2 to aid their own expression.

The amount of protein (hormone, enzyme, or transcription factor) made by a gene in a cell determines how much the gene is expressed in that cell. In adult type GCT, the FOXL2 gene is mutated, which produces a mutant form of its transcription factor. If the transcription factor is abnormal, it will probably not bind to genes that it is meant to and/or bind to genes that it normally wouldn't. This will cause a difference in the expression of FOXL2 controlled genes. In a juvenile GCT, however, FOXL2 is not mutated but expression is significantly reduced which means that, for some reason, less FOXL2 transcription factor is being synthesized. Less FOXL2 transcription factor also means less protein from genes with FOXL2 transcription factor binding sites. So, in a GCT not only is the gene FOXL2 acting differently because of a mutation in adult GCT or an under-expression in juvenile GCT, but other genes' expressions may also be affected. And while we can see a mutation in FOXL2 in adult GCT and can place some direct blame on it for tumour genesis, juvenile GCT is more of a puzzle, since the FOXL2 itself is not the source of the problem, but one of the many proteins that comes in contact with it is.
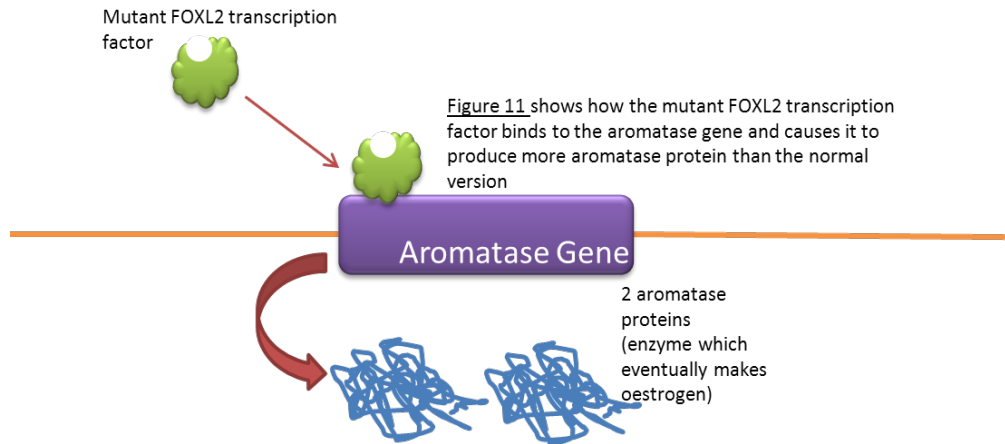
Currently, scientists know of four genes (and are investigating many more) for which FOXL2's transcription factor is necessary for its protein production, but only two of those are active granulosa cells. One of the two genes is called aromatase – its protein is the enzyme that helps to make the

commonly known hormone oestrogen. Therefore, normal, functional FOXL2 is essential for ovarian cells, as oestrogen would not be produced in the proper quantities. Every time a new piece of FOXL2 transcription factor enters the gene's binding site, more aromatase protein is formed (Figure 10). The more transcription factors there are floating around, the higher chance that a binding site will be occupied more than once. The opposite would also be true.

FOXL2 transcription factor

Figure 10 shows how the mutant FOXL2 transcription factor binds to the aromatase gene and causes it to produce aromatase protein

Aromatase Gene

1 aromatase protein (enzyme which eventually makes oestrogen)

In adult GCT, a mutant FOXL2 gene means that a mutant form of its transcription factor is made inside the cell. Researchers at the Granulosa Cell Tumour Foundation of New Zealand are trying to figure out how the mutant FOXL2 protein acts differently from its normal prototype (called the wildtype version) by viewing their effects on genes side-by-side. They are unsure how exactly the mutant FOXL2 interacts with the genes that normal FOXL2 is supposed to regulate – it may still work with genes containing typical FOXL2 binding sites but cause them to produce more or less protein per transcription factor than the regular strand of FOXL2 would (Figure 11). The FOXL2 mutation may also cause it to misidentify the buddy genes it is supposed to control and bypass them. Mutant forms might also fit into a wider variety of binding sites and therefore control different genes to the normal form, and therefore, many new proteins may be made inside the cell.

Mutant FOXL2 transcription factor

Figure 11 shows how the mutant FOXL2 transcription factor binds to the aromatase gene and causes it to produce more aromatase protein than the normal version

Aromatase Gene

2 aromatase proteins (enzyme which eventually makes oestrogen)

Because FOXL2 most likely acts as a tumour suppressor, mutation or lost expression of this gene may lead to a higher rate of cell division and risk of tumour development. Also, if a mutant strand of FOXL2 transcription factor is able to bind to a gene that regular FOXL2 is not, the cell may begin to produce a lot of new proteins that it does not normally make and may change its function as a result. This may have many different repercussions, stimulated by the foreign products of a gene incorrectly activated by the mutant transcription factor. Another negative consequence of mutant FOXL2 is that it may have the ability to change the concentrations of proteins made by FOXL2 transcription factor-compatible genes (Figure 11), and constantly elevated or depleted levels of normal ovarian protein may also cause undue stress on granulosa cells. All of these plausible situations could lead to the creation of a GCT.

ESR2 and Oestrogen Receptor Beta

ESR2 is a gene found on Chromosome 14 that produces oestrogen receptor beta (ERbeta) (Figure 12). This gene and its protein are crucial for female organs such as the breasts, uterus, and ovaries and is also involved in some male reproductive, cardiovascular, nervous, and immune system functions.

Figure 12 shows the location of the Oestrogen Receptor Beta (ERB) gene on chromosome 14 at the red arrow.

If you understood the FOXL2 section, these paragraphs will be a breeze since the protein synthesized by the ESR2 gene, the ERbeta protein, is also a transcription factor. The only difference

between FOXL2 protein and ERbeta protein is ERbeta requires a partner, called a ligand, for it to be activated and do its job. This is why it is called a "ligand-induced transcription factor."

A ligand is basically a hormone – ERbeta protein's partner ligand is, obviously, oestrogen. As oestrogen is produced in the female body, certain percentages of it are appropriated for different uses. Some oestrogen seeks out and connects with the ERbeta protein in order to create the ERbeta transcription factor (remember, transcription factors are important because they bind to other genes and force them to produce proteins of their own) (Figure 13). The ERbeta transcription factor can only aid genes with which it is compatible, just like the principles behind FOXL2's transcription factor.
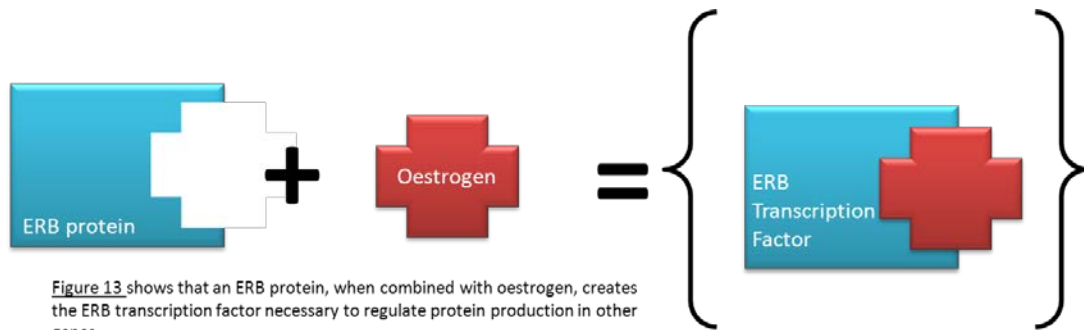

Figure 13 shows that an ERB protein, when combined with oestrogen, creates the ERB transcription factor necessary to regulate protein production in other genes.

ERB transcription factors "read" genes to find a certain pattern of DNA bases that will accept it. The specific pattern of bases that the ERB transcription factor looks for is called the Oestrogen Response Element (ORE) and only certain genes contain one. Once the ERbeta transcription factor finds a gene with a ORE, a part of the ERbeta transcription factor called a zinc finger binds itself to the gene which then enables a change in gene expression.

For learning purposes, let's say that the ORE is the DNA base sequence AATCG, meaning that any gene containing this exact 5-base sequence somewhere in its code is a gene controlled by an ERbeta transcription factor (the ERbeta protein plus oestrogen). This would imply that the zinc finger in the ERbeta transcription factor likes to bind to this section of DNA (AATCG). Once the zinc finger in the ERbeta transcription factor lines up with the ORE, the gene with the ORE makes its own protein (Figures 14 and 15).
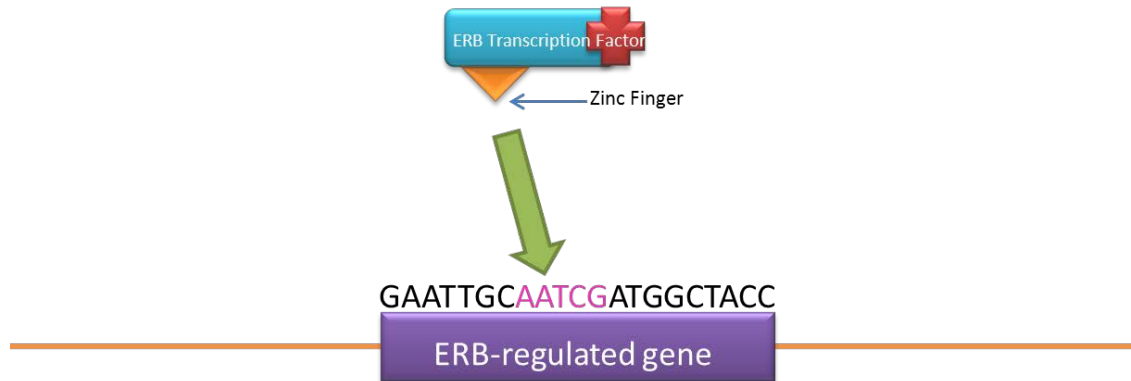
GAATTGCAATCGATGGCTACC

ERB-regulated gene

Figure 14 demonstrates how an ERB transcription factor recognizes a gene with which it is compatible. If AATCG (the HRE in this example) is present in any gene, then the ERB transcription factor, which always has an HRE-compatible zinc finger, will bind to it.

Figure 15 demonstrates how an ERB transcription factor recognizes a gene with which it is compatible. If AATCG (the HRE in this example) is present in any gene, then the ERB transcription factor, which always has an HRE-compatible zinc finger will bind to it. The gene then makes proteins.

GAATTGCAATCGATGGCTACC

ERB-regulated gene
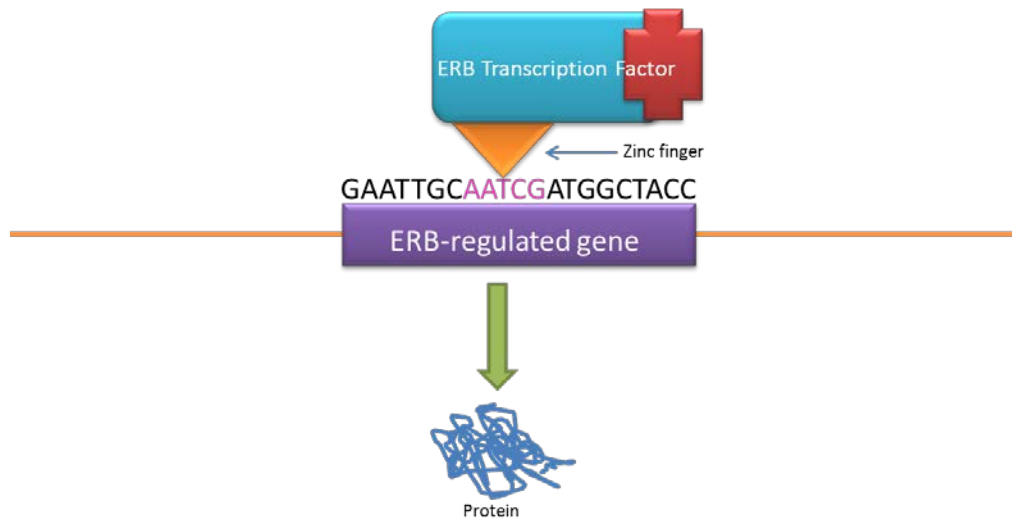
Protein

The ESR2 gene (as in the gene that actually produces the ERbeta protein) is thought to be a tumour suppressor because an inverse correlation can be identified between severity of tumour and level of ERB expression in breast, colon, and ovarian cancers. In ovarian cancer, when cells are forced to over-express their ESR2 gene, they have reduced rates of cell division. Therefore, the more ERbeta protein there is in a cell (meaning that ERbeta is over-expressed), the less likely the tumour will grow out of control. Knocking down the effects of ERbeta, logically, increases your risk. A recent study of ERbeta levels and stage of tumour showed that women with higher stage epithelial tumours had less ERbeta expression than those with Stage 1 disease. So, a drug that could increase the expression of ERbeta in ovarian cells could reduce the risk of advanced stage ovarian cancer. Why is this the case? Oestrogen is usually proliferative in ovarian cells, which means that it causes cells to divide, but this is when

oestrogen is acting through a different oestrogen receptor called oestrogen receptor alpha (ERalpha). The current thinking is that ERalpha and ERbeta compete with each other, but the more ERbeta protein there is in the cell to bind with oestrogen and to channel it to reduce cell growth, the less oestrogen there is freely roaming to provoke cell growth through ERalpha.

The popular drug tamoxifen works in conjunction with the ERalpha and ERbeta. Referring back to Figure 13, oestrogen binds to ERbeta or ERalpha protein to make transcription factor. Tamoxifen works to bind with the ERalpha and ERbeta proteins before oestrogen can. In ERalpha, this is an excellent thing – if oestrogen binds with the ERalpha protein, it can encourage cell growth; if tamoxifen gets to ERalpha protein before it does, cell growth in inhibited. However, tamoxifen cannot differentiate between ERalpha (the bad protein) and ERbeta (the good protein), so sometimes tamoxifen can keep the ERbeta from carrying out its positive role in the body, though it is quite effective in shutting down ERalpha.

A medicine that focuses on increasing ERbeta (and not ERalpha) could also help reduce the chances of recurrence in newly diagnosed patients. ERbeta can affect cell migration in certain organs; the more ERbeta present, the less cell movement there is. In theory, an ERbeta targeting drug would be able to eliminate, or at least contain, the spread of cancerous cells from a ruptured tumour into other organs or the abdominal region.

ERbeta expression is relatively high in some GCTs (meaning that the cell is producing more ERbeta protein), despite the fact that elevated ERbeta levels are typically associated with reduced risk of cancer. However, this may be the reason that GCTs are slow-growing and classified as a low-grade malignancy. There have been a few documented cases where GCTs were ERbeta-negative (meaning little or no ERbeta expression at all), and the women diagnosed with those tumours either died or recurred within 5 years of initial diagnosis. There is also the possibility that ERbeta expression can reduce over time, leading to recurrence.

Therefore, identifying and correcting the underlying level of ERbeta expression in GCT, and then restoring ERbeta's job as a tumour suppressor may be a sensible treatment. A combination of the repercussions of the over-expression of the ESR2 gene itself and the over or under stimulation of the genes it rules may create the perfect environment for ovarian cancer and GCT.

<u>Conclusions</u>

Granulosa cell tumour (GCT) is a rare form of ovarian cancer but the predominant type of sex-cord or stromal tumour. Very little is known about its treatment or management in the medical community due to its rarity; however, scientists within the last few years have taken more interest in it. While it is technically a low-grade, slow growing malignancy, blanket treatments administered to GCT patients tend to not work as effectively, and the lack of proper follow-up leads to the 80% fatality rate of patients with recurrent GCT. A GCT specific treatment plan would significantly heighten survival rates across the board, since GCT caught in early stages and removed without complications has a fantastic 90% long-term survival rate.

There are a few genes and proteins that are of particular scientific interest: FOXL2 and Oestrogen Receptor Beta (ERbeta), both of which are abnormally expressed in GCT cell samples. FOXL2 is mutated and under-expressed and ERbeta shows reduced expression. Researchers experiment with different levels of each of these substances to see how cancerous and benign cells respond, always trying to find a point in the cell that is vulnerable to attack and therefore reducing tumour development. Further investigation into the role of these genes in the malignant development of granulosa cells is necessary to invent effective treatments to combat GCT. Seemingly, medications created to increase the expression of the FOXL2 and ERbeta transcription factor would help ward off recurrence; genes highlighted as GCT initiators could also be screened as an early marker for detection, and could determine a woman's risk of developing the cancer again in the future.

And while there has been an exponential increase in the general understanding and awareness of GCT, especially in the last few years, we still have a long way to go until a possible cure is devised. Luckily, there is always a group of passionate scientists, doctors, and students who enthusiastically spend their time studying this confusing disease and all its peculiarities. The future is bright, and I am hopeful.