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Genetic Mosaicism: Introduction and Applications

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Abstract— This paper sheds light on the clinical applications of Genetic Mosaicism. The recent advancements in creating mosaics (genetically different cell types arising from a single zygote) and chimeras (from more than one zygote) in a lab environment can be extraordinarily useful in the study of biological systems, and can be created intentionally in many model organisms in a variety of ways. The paper explains how this phenomenon can be used to study gene functions, modelling of genetic disorders, understand embryonic development and evaluate therapeutics. It also focuses on how this technique is a better evaluation tool than the conventional genetic testing methods.

Keywords— Chimerism, Somatic Mosaicism, X-inactivation, Tetragametic Mosaicism, Tortiseshell cat, Gene Autonomy, Epigenetics.

Introduction- The phenomenon of genetic mosaicism denotes the presence of two or more populations of cells with different genotypes in one individual who has developed from a single fertilized egg. [10]

It was discovered by Curt Stern in the 1930s. He demonstrated that genetic recombinations (that usually takes place in meiosis) can also take place in mitosis resulting in somatic mosaics. These are organisms which contain two or more genetically distinct types of tissue. The term somatic mosaicism was used by C.W. Cotterman in 1956 in his seminal paper on antigenic variation.

A. Mosaics and Chimeras: A Comparison

Mosaics may be contrasted with chimerism, where two or more genotypes arise from the fusion of more than one zygote in the early stages of embryonic development. Mosaics are common; in fact, roughly half of the mammals on earth are a type of mosaic. A chimera can only be acquired as a result of an embryology experiment.

B. Types of Mosaicism

Different types of mosaicism exist, such as gonadal mosaicism or somatic mosaicism.
Somatic mosaicism occurs only in the somatic cells when different genotypes arise from a single fertilized egg cell, mostly due to mitotic errors at first or later cleavages. In rare cases, intersex conditions can be caused by mosaicism where some somatic cells in the body have XX and others XY chromosomes. The most common form of somatic mosaicism is encountered in trisomies, when only a selection of cells is affected by non-disjunction. (46/47 XY/XXY). [2][10]

Revertant somatic mosaicism is a rare recombination event in which there is a spontaneous correction of a mutant allele. Gonadal mosaicism or germline mosaicism is a special form of mosaicism, where some gametes, i.e. either sperm or oocytes, carry a mutation, but the rest are normal. [9]

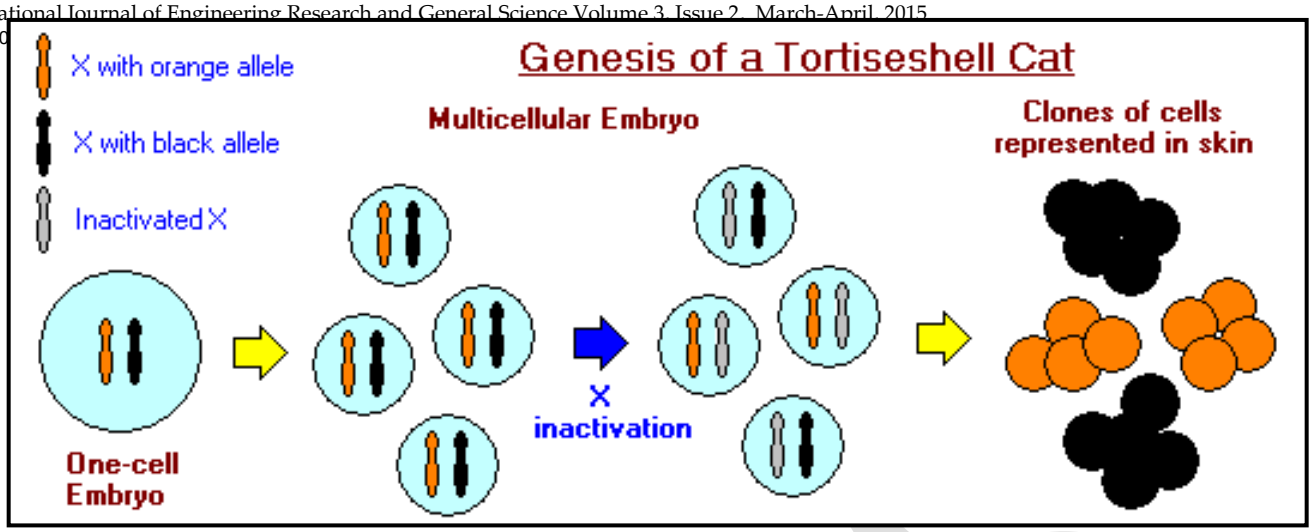


Fig.1 X-chromosome inactivation mosaicism in Tortiseshell cat

Mechanisms- A genetic mosaic is a creature whose body is built of a mixture of cells of two or more different genotypes. In mammals they arise by several different mechanisms:

A. Placental Exchange

The sharing of blood supplies by separate embryos. This occurs with the occasional fraternal cattle twins and also —less often — with human fraternal twins who have shared the same placenta. Blood stem cells of each twin seed the bone marrow of the other. Only their blood cells are mosaic.

B. Mitotic Errors

During early development, errors during mitosis can produce stem cells that go on to populate a tissue or organ with, for example, a chromosomal aberration (e.g., aneuploid).

Example: Occasionally a baby is born with blood cells that have three copies of chromosome 21 (the same set responsible for Down syndrome). This can produce a leukemia-like illness that, fortunately, often disappears as that cell population declines. [5]

C. X-inactivation

All female mammals are mosaic for the genes on the X chromosome because of the random inactivation of one or the other X chromosome in all their somatic cells.

D. Cancer

Anyone having a cancer is a genetic mosaic because all cancers are made up of the descendants of cells carrying a suite of mutations not found in normal cells.

E. *Mitotic Mutations*

Recent advances have enabled the coding portions of the genome of single cells to be sequenced. Early results indicate that even normal cells in an adult have accumulated a suite of somatic mutations that differs from cell to cell. However, the rate of somatic mutations in these normal cells is only a fourth of that in cancer cells. All multicellular organisms are likely to be somatic mosaics to some extent. Since the human intergenerational mutation rate is approximately 10−8 per position per haploid genome and there are 1014 cells in the human body, it is likely that during the course of a lifetime most humans have had many of the known genetic mutations in our somatic cells and thus humans, along with most multicellular organisms, are all somatic mosaics to some extent. To extend the definition, the ends of chromosome 'telomeres', shorten with every cell division and can vary from cell to cell thus representing a special case of somatic mosaicism. [2] [4]

X-Inactivation Mosaicism- To compensate for the presence of only one X-chromosome in male cells, compared to female cells, one of the two active X-chromosomes in every cell of the female blastocyst is randomly and stably inactivated. However, this cannot be said to be true mosaicism since, X-inactivation is not maintained, within cells of the female germ line. The inactive X-chromosome of each female germ cell is reactivated at the oogonium stage. Both X-chromosomes then remain active throughout

meiosis and development of the definitive oocyte. Thus, male zygotes inherit an active X-chromosome from the oocyte (which remains active throughout the life-time of the male), and a Y chromosome from the spermatozoon. On the other hand, female zygotes inherit two active X-chromosomes, one from the oocyte and one from the spermatozoon. Both X-chromosomes remain active in all cells through early cleavage until random, stable inactivation of one of them in each cell occurs again in the late blastocyst. The consequence of X-inactivation in cells of the female blastocyst is that their clonal descendants differ with respect to whether the paternal or maternal X-chromosome remains active and thus, whether they express specific maternal or paternal genes. The classical example of this phenomenon is the female calico cat which inherits an X-linked yellow allele from one parent and an X-linked non-yellow allele from the other. One or the other colour is expressed in patches which represent clones descending from cells with the respective active X-chromosome.

A. Occurrence in cats

Cats provide a unique opportunity to observe X-chromosome inactivation and help visualize how it affects all females. Tortiseshell cats, as seen below, have a coat that is a mixture of black and orange hair. Calico cats are similar, but also have patches of white, which is encoded by another gene.

The gene encoding orange coat color is X-linked (that is, on the X chromosome). Black color is encoded by either a co-dominant allele on the X chromosome or, more likely, an autosomal gene that is masked by the orange gene. For explanatory purposes, we will consider the orange gene (O) and its non-orange allele (o), to both be X-linked. Normal male cats have a single X chromosome and can carry either the O or o gene, leading them to have an orange or black coat, respectively.

Female cats, with two X chromosomes, can have any of three genotypes relative to the orange gene: OO (orange coat), oo (black coat) or Oo (tortiseshell or calico). The tortiseshell pattern of fine patches of black and orange reflects the pattern of X chromosome inactivation in the hair follicles, as shown in Fig. 1.

Fig. 2 Venus the Mosaic Cat

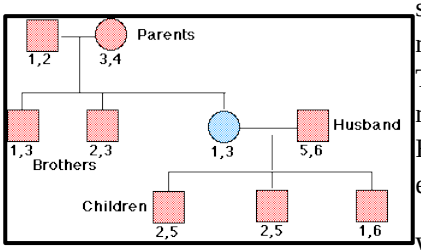
In black patches, the X chromosome bearing the orange allele has been inactivated and the X chromosome bearing the non-orange allele is active. Precisely the converse is present in patches of orange fur. The random nature of X-chromosome inactivation is evident - there are relatively large patches of both black and orange, but most of the coat is a fine mixture of orange and black. Fig. 2 depicts how X chromosome inactivation leads to unevenly coloured patches.

The pattern of X chromosome inactivation seen as black and orange fur in the coat of a tortiseshell cat is present in all tissues of all female mammals. That pattern is just not usually visible because, for example, human skin colours are not encoded by X-linked genes. However, understanding X chromosome inactivation and mosaicism is of great importance in all species for understanding the pathophysiology of X-linked genetic diseases. [1]

Existence of Mosaicism- Genetic Mosaics since their discovery have solved many mysteries of human physiology and have contributed to better healthcare. A few examples are as follows:

A. A. Tetragametic Human Mosaic

In 2002 there was a discovery of a tetragametic woman- that is a woman derived from four different gametes, not just two. Since she needed a kidney transplant, the following tests were performed leading to various results. [4]



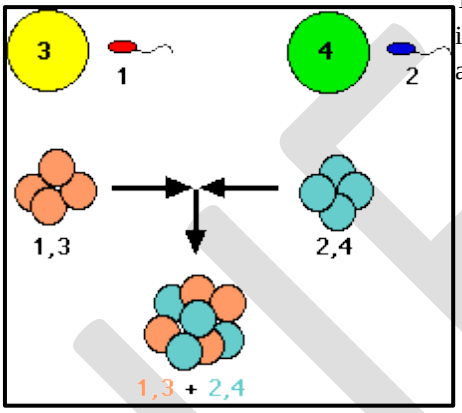
Tissue typing, which is done with blood cells, showed her to have inherited the "1" HLA region of her father (who was 1, 2) and the "3" region of her mother (who was 3, 4). See Fig. 3. Her husband was typed 5, 6. Of her three sons, one was 1, 6 which was to be expected, but the other two were both 2, 5.

When tissue typing was done for other tissues like skin, hair, thyroid, bladder, and cells scraped from inside her mouth revealed that DNA of not only 1 and 3 but also 2 and 4 existed in her body.

Fig. 3 Tissue Typing Results

The woman can accept a kidney from any one of her brothers as well as her parents without fear of rejecting it since she has all four sets of transplantation antigens. The HLA region on chromosome 6 carries a set of genes that encode the major transplantation antigens; that is, the antigens that trigger graft rejection. Ordinarily, there is only a 1 in 4 chance that two siblings share the same transplantation antigens if both parents were heterozygous as in her case.

This was possible because mostly her mother had simultaneously ovulated two eggs one containing a chromosome 6 with HLA 3; the other with HLA 4. Her father would, of course, have produced equal numbers of 1-containing and 2-containing sperm. Soon thereafter the resulting early embryos fused into a single embryo. As this embryo developed into a fetus, both types of cells participated in constructing her various organs including her oogonia (but not, apparently, the blood stem cells in her bone marrow). Although she was a mosaic for the HLA (and other) genes on chromosome 6, all her cells were XX. So both the father's successful sperm cells had carried his X chromosome. Refer Fig.4.



Tetraparental humans have been found that were mosaic for sex chromosomes as well; that is, some of their cells were XX; the other XY. In some cases this mosaic pattern results in a hermaphrodite — a person with a mixture of male and female sex organs.

Fig. 4 Tetraparental cells

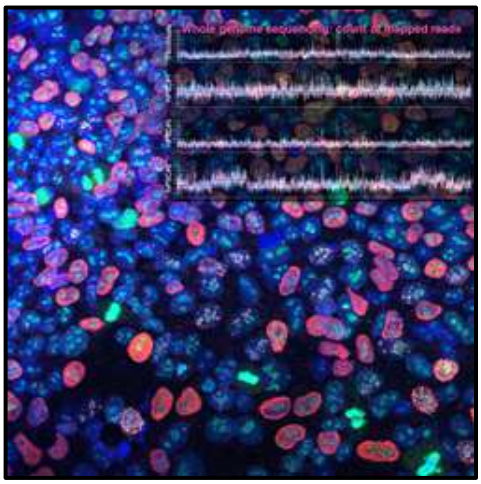
B. Skin cells reveal DNA's genetic mosaic

The study paves the way for assessing the extent of gene variation, and for better understanding of human development and disease. 30% of skin cells harbor copy number variations (CNV), which are segments of DNA that are deleted or duplicated. The mosaic seen in the skin could also be found in the blood, in the brain, and in other parts of the human body.

Whole genome sequencing was used to study induced pluripotent stem cells lines (iPS), (which are stem cells developed from a mature-differentiated cell). Cells taken from the inner upper arms of two families were grown. Characterization of these iPS cell lines and comparison to the original skin cells was done.

While observing that the genome of iPS cells closely resembles the genome of skin cells from which they originated, several deletions or duplications involving thousands of base pairs of DNA were identified. Additional experiments to understand the origin of those differences were performed, which showed that at least half of them pre-existed in small fractions of skin cells. These differences were revealed in iPS cells because each iPS line is derived from one, or very few, skin cells. Refer Fig. 5.

The observation of somatic mosaicism has far-reaching consequences for genetic analyses, which currently use only blood samples. When we look at the blood DNA, it's not exactly reflecting the DNA of other tissues such as the brain. There could be mutations that we're missing.



Many other examples of genetic chromosomal mosaicism occur in humans. Nonetheless, the most common involve sex chromosomes (XO/XX, XX/XY, XXX/XX, XY/XO). Indeed, a high proportion of Turner syndrome individuals are mosaics.

Fig. 5 Skin cells showing Mosaicism

Uses of Genetic Mosaics-

A. To study gene function in stem cells

Genetic mosaics can be extraordinarily useful in the study of biological systems, and can be created intentionally in many model organisms in a variety of ways. They often allow for the study of genes that are important for very early events in development, making it otherwise difficult to obtain adult organisms in which later effects would be apparent.

B. To determine gene autonomy and to study epigenetics

They can be used to determine the tissue or cell type in which a given gene is required and to determine whether a gene is cell autonomous. That is, whether or not the gene acts solely within the cell of that genotype, or if it affects neighbouring cells which do not themselves contain that genotype, but take on that phenotype due to environmental differentiation.

C. To analyse complex systems

Mosaics are routinely used to investigate cell lineages, patterns of growth and gene function, and provide a means to clear analytical hurdles that otherwise limit standard genetic approaches. They are employed as a means to test whether genes act cell autonomously or non-autonomously in different tissues and to dissect tissue-tissue interactions in less tractable, complex systems. [6]

D. In genetic engineering

Mosaics can be used in somatic or germline cell therapy. The specific organs and tissues can be targeted to correct a mutation or provide a new function in human cells. It holds the promise of treating genetic disorders. Any genetic modification in somatic cells is not passed on to the progeny. It has been successfully used to treat multiple diseases, including X-linked SCID (severe combined immunodeficiency), chronic lymphocytic leukemia and Parkinson's disease. Germline cell therapy could treat congenital disorders but has not been attempted on humans as yet.

Recent Mosaicism Studies-

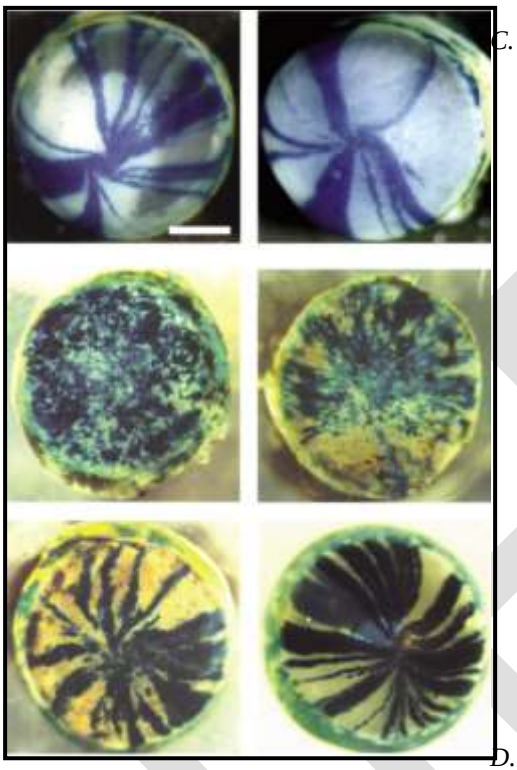
A. In Fruit Flies and Drosophila

Genetic mosaics are a particularly powerful tool when used in the commonly studied fruit fly, where they are created through mitotic recombination. Mosaics were originally created by irradiating flies heterozygous for a particular allele with X-rays, inducing double-strand DNA breaks which, when repaired, could result in a cell homozygous for one of the two alleles. After

further rounds of replication, this cell would result in a patch, or "clone" of cells mutant for the allele being studied. More recently the use of a transgene incorporated into the Drosophila genome has made the system far more flexible. [11]

B. In nematode Caenorhabditis elegans

In Caenorhabditis elegans, genetic mosaics have been generated, identified, and analysed for the purpose of defining where in the animal a gene of interest must be expressed to cause a particular phenotypic effect. Most C. elegans mosaics that have been studied were homozygous mutant for a recessive mutation in all cells and carried additionally, in some cells, one or more copies of the wild-type allele of the same gene on an extrachromosomal element. Before mosaic analysis is undertaken, a mutant phenotype corresponding to the absence of the wild-type gene from all cells is analysed and described. Some mutant phenotypes are described in whole-animal terms, such as uncoordinated movement, longer-than-normal lifespan, abnormal body shape, or inviability. Such traits can be analysed in mosaic animals. The overall phenotypes are recorded for mosaic animals in which it is known which cells carry the wild-type gene and which do not, with the goal of establishing which cells must carry the wild-type gene to prevent the appearance of the mutant phenotype. The responsible cell or group of cells is referred to as the anatomical focus of the action of the gene with respect to the phenotype under study. [7]



C. C. In Mice for testing Mosaicism in Mammalian Eyes

Analysis of experimental mouse mosaics provides a means of investigating patterning and differentiation within the developing mammalian eye. Mosaic mice carry two or more genetically distinct cell populations and extend the repertoire of analytical tools available to the geneticist. Here we review the impact these techniques have had on our understanding of eye organogenesis. Mosaics are routinely used to investigate cell lineages, patterns of growth and gene function, and provide a means to clear analytical hurdles that otherwise limit standard genetic approaches. Mouse X-inactivation mosaics can be generated easily by appropriate genetic crosses. The first useful X-linked cellular marker was Is(In7;X)1Ct (Cattanach's translocation), resulting from the insertion of an inverted piece of chromosome 7 into the X chromosome (Cattanach, 1961). The inserted length of chromosome 7 includes the wild-type C allele of the albino locus. Homozygous albino female mice (c/c) that are hemizygous for the Is(In7;X)1Ct insertion have variegated coat and eye pigment. Refer Fig. 6. [3]

Fig.6 Mosaicism in mice cornea

D. In maize

Perhaps the first observed occurrences of Mosaicism were in Zea mays- Maize when Barbara McClintock discovered the phenomenon of jumping genes that led to mottling in maize kernels. She noticed insertions, deletions, and translocations, caused by these elements. These changes in the genome could, for example, lead to a change in the color of corn kernels. About 85% of the genome of maize consists in TEs which results in the varied colouring and patterns in the kernels. On comparing the chromosomes of the current generation of plants and their parent generation, she found certain parts of the chromosomes had switched positions on the chromosome. She disproved the popular genetic theory of the time that genes were fixed in their position on a chromosome. She found that genes could not only move, but they could also be turned on or off due to certain environmental conditions or during different stages of cell development. She also showed that gene mutations could be reversed. Refer Fig. 7. [8]

Fig.7 Mosaicism due to TE's in maize

Conclusion- The frequencies of chimerism and mosaicism are unknown, but doctors might benefit from a better understanding of both conditions. In recent years, tantalizing hints have emerged that pockets of genetically mismatched cells may contribute to conditions as common as infertility, autism and Alzheimer's disease.

Personalised medicine is the future of clinical technology. And if chimeras and mosaics are more common than we realize, they will complicate future efforts to tailor drug treatments to people's individual genetic constitutions. Two genetically different tissues in one body might produce an unpredictable response to a drug. Hence, it becomes essential to study Mosaicism in detail in humans to formulate new medical technologies and drugs so that their efficacy can be optimized and drugs can be devised specifically.

It is speculated that Mosaicism could be used to change physical appearance, metabolism and even improve physical capabilities and mental faculties like memory and intelligence although for now these uses are limited to science fiction.

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