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Hox Genes: Literature Review and Clinical Overview of Human Defects and Cancer

Christopher Ellbogen

University of Wyoming, cellbog2@uwyo.edu

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HOX GENES:
LITERATURE REVIEW AND CLINICAL OVERVIEW
OF HUMAN DEFECTS AND CANCER

Chris Ellbogen

Senior Honors Project

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Hox Genes: Literature Review and Clinical Overview of Human Defects and Cancer

Introduction to Genes

While most people are familiar with DNA, or deoxyribonucleic acid, few actually understand how the language of DNA translates to the differences in traits or body formation that we see every day. A gene is a sequence of DNA nucleotides: Adenine, Cytosine, Thymine, or Guanine. These nucleotides encode for specific traits and features. Distinct sections of nucleotides are read and turned into a chain of RNA, ribonucleic acid, through a process known as transcription. Through another process called translation this chain of RNA nucleotides is used to create proteins, which are an essential building block for living things. Genes code for every genetic difference between species and individuals, from blue eyes to skin color in humans to the color and number of petals in flowers.

These genes are arranged on chromosomes, which are packaged DNA within a cell's nucleus. The genes are passed from one generation to the next via a complex mechanism that duplicates the individual nucleotides. Very rarely a mistake in this process naturally occurs, resulting in a switch between an A, C, T, or G base pair. These are called mutations, and they can either be meaningless or have extreme consequences. Through other processes sometimes entire sections of DNA are copied or duplicated.

Hox Genes

Thomas Hunt Morgan, who received the Nobel Prize in Physiology or Medicine in 1933 for discovering the role of genes in heredity, first theorized that there were genes that regulate body formation. More simply put, he was the first to think about how bodies know where to place certain features or appendages within the context of an individual's genetic code. In his genetic experiments Morgan used the common fruit fly, *Drosophila melanogaster*, as his model organism. His "fly lab" held tens of thousands of fruit flies, which were used in place of more complicated animals to understand the science of genetics.¹ *Drosophila* have a nine day development, which is fast enough to be able to track genetic changes over dozens of generations. Their small size and simple diet allowed for thousands, if not millions, to be inexpensively maintained in his lab. Fruit flies can also be seen under a microscope easily, which makes them a more appealing alternative to much smaller microscopic organisms. It is ethical to experiment on these animals compared to more complex organisms because of their numbers and simple nature.

Drosophila may seem to have nothing in common with humans, but as animals their genetic code is remarkably similar. There are many human homologues, or comparable genes, within the *Drosophila* genetic code.² Most research done on fruit flies

¹ "Thomas Hunt Morgan – Biographical," The Official Website of the Nobel Prize, accessed April 26, 2016, http://www.nobelprize.org/nobel_prizes/medicine/laureates/1933/morgan-bio.html.

² Dow, Julian AT (March 2012) *Drosophila* as an Experimental Organism for Functional Genomics. In: eLS. John Wiley & Sons, Ltd: Chichester.

can be replicated in humans, and so to this day *Drosophila* is still one of the most popular model organisms for researchers.

Though Thomas Hunt Morgan was unable to identify which genes controlled for the body plan, decades later three scientists would complete his work. In 1995 Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Wieschaus shared the Nobel Prize in Physiology or Medicine for their discovery of the 39 genes which code for the human body plan. These 39 Hox genes, as they were called, are an incredibly small amount of the 20,000 to 25,000 estimated genes in the human genome. Though few in number these genes have a huge impact on how the human body is arranged, and any abnormality in their genetic code can have drastic consequences.

Like Thomas Hunt Morgan, Nüsslein-Volhard and Wieschaus relied on *Drosophila* extensively in their research. Nüsslein-Volhard, and Wieschaus worked together in a German lab combing through all 5,000 *Drosophila* genes through the selective breeding of 40,000 fruit fly families. They were eventually able to narrow the range of genes responsible for body plan by deliberately inducing mutations into the *Drosophila* genome.³ This range is labelled the homeobox, from the Greek word ‘ομοιος’ meaning “‘alike.’”⁴ Genes within the homeobox that code for the placement of specific features are shortened to the term Hox genes.

DOI: 10.1002/9780470015902.a0000561.

³ Nüsslein-Volhard, Wieschaus. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature*. 287(5785):795-801.

⁴ Goodman. 2002. Limb Malformations and the Human HOX Genes. *American Journal of Medical Genetics*. 112:256-265.

Edward Lewis, a professor at the California Institute of Technology, discovered colinearity within Hox genes, which is the unique phenomena where the order of the genes on the chromosome are the same as the order along the body axis.⁵ For example, the genes in *Drosophila* that code for antennae are positioned at the beginning of the homeobox sequence, while the gene that codes for legs are closer to the end. This is known as spatial colinearity. Sometimes, however, genes at the anterior end of some Hox gene clusters are expressed earlier in ontogeny (development) than those at the posterior end, which is temporal colinearity. In even fewer cases, genes proximal to a specific enhancer are expressed at higher levels than those more distal, which is quantitative colinearity.⁶

Interestingly, there are many exceptions to this rule. Sometimes Hox genes are grouped in clusters that violate the general trend of spatial colinearity. Pentaradial echinoderms, which have five identical body parts around a central point of symmetry, curiously express no temporal colinearity. Urochordates (sea squirts) exhibit no colinearity within their genetic code whatsoever.⁷ Colinearity has been a unique defining feature of Hox genes, but as further research is conducted it seems as though its presence is more of a guideline than a rule.

Hox genes are also important because they are evidence to how we know that every cell carries in its DNA all of the information necessary to build the entire organism.

⁵ Monteiro, Ferrier. 2006. Hox genes are not always Colinear. *International Journal of Biological Sciences*. 2(3):95-103.

⁶ Monteiro, Ferrier; 95.

⁷ Lemons, McGinnis. 2006. Genomic Evolution of Hox Gene Clusters. *Science*. 313(5795):1918-1922.

This was determined through an induced mutation in the Antennapedia HOM-C (homeotic complex) regulatory region in *Drosophila* that caused the fruit fly's antennae to be replaced by a set of legs in development.⁸ Antennae precursor cells in a normally-developing fruit fly must therefore contain both antennae and leg DNA to be able to differentiate into either. Therefore if any group of cells can be induced to differentiate into any structure, then every cell should carry the genetic code for the entire organism instead of just that one part.

Hox Genes in Evolution

Hox genes are also important in furthering human understanding of evolution. Evolution is essentially descent with modification, and accidental Hox gene duplication created evolutionary novelties. Through random mutation, Hox genes were sometimes expanded or duplicated, which played a large role in species differentiation. For example, the human homeobox is made up of 39 total Hox genes spread out over four parallel clusters resulting from duplication events.^{9 10} These clusters are designated A through D, and they are used to name each Hox gene. Duplicating Hox genes also duplicated the specific body structures that they code for, and so it is possible that the repeating identical

⁸ Grier, Thompson, Kwasniewska, McGonigle, Halliday, Lappin. 2005. The pathophysiology of HOX genes and their role in cancer. *Journal of Pathology*. 205:154-171.

⁹ Goodman, 256.

¹⁰ Wagner, Amemiya, Ruddle. 2003. Hox cluster duplications and the opportunity for evolutionary novelties. *Proceedings of the National Academy of the Sciences of the United States of America*. 100(25):14603-14606.

segments in animals such as insects derive from these duplication events. For example, millipedes have hundreds of repeating leg segments, and it is possible that this arose from Hox gene duplication.

Because Hox genes control for so much, their structure is evolutionarily conserved.¹¹ Mutations are usually lethal, which is to say that the random mistakes in copying nucleotides leads to an error so problematic that it causes the death of the organism. A dead organism cannot reproduce, and so over the course of millions of years most Hox genes remain relatively unchanged, even between drastically different species. To demonstrate this a Mouse Hox-1.3 gene can be inserted into the *Drosophila* genome to replace the *sex combs reduced* gene.¹² This substitution of one Hox gene for another results in a functionally equivalent organism, which means that despite their phylogenetic separation the Hox genes of the two species are remarkably similar.

On the rare occasion that a Hox gene mutation is not lethal in animals there will usually be extreme defects. The most common example is the replacement of antennae with legs in *Drosophila* as mentioned earlier. Other mutations in fruit fly Hox genes can lead to an extra pair of wings or other body segment repetition.

¹¹ Hart, Awgulewitsch, Fainsod, McGinnis, Ruddle. 1985. Homeo Box Gene Complex on Mouse Chromosome 11: Molecular Cloning, Expression in Embryogenesis, and Homology to a Human Homeo Box Locus. *Cell*. 43(1):9-18.

¹² Zhao, Lazzarini, Pick. 1993. The mouse Hox-1.3 gene is functionally equivalent to the *Drosophila* Sex combs reduced gene. *Genes & development*. 7(3):343-54.

Human Hox Gene Abnormalities

Non-lethal mutations in the *Drosophila* Hox genes are drastic, such as an additional pair of wings or the replacement of body parts. Comparatively, non-lethal human mutations are nowhere near as extreme. Further research should be conducted to try to determine why human Hox gene mutations do not replicate entire segments or appendages as mutations in *Drosophila* do.

A mutation in the HOXA13 gene causes hand-foot-genital syndrome (HFGS). This syndrome is characterized by malformation of the distal limbs (See Figure 1) and of the lower urogenital tract. This rare condition is dominantly inherited, and so the loss of a single functional copy of the HOXA13 gene can cause HFGS.¹³

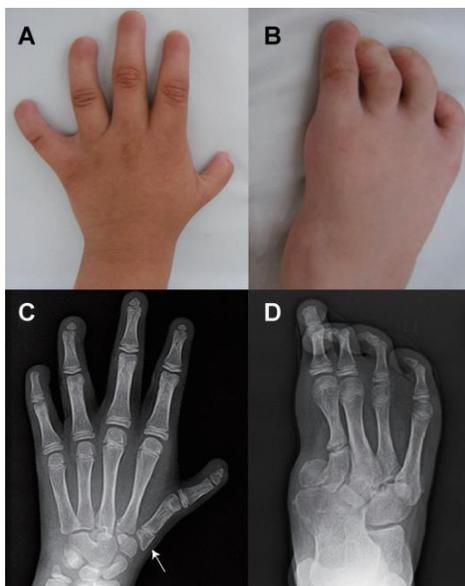


Figure 1: Hand Foot Genital Syndrome (Source: Imagawa, Kayserili, et al., 2014)

¹³ Grier et al., 159.

Similarly, a specific mutation in the HOXD13 gene causes synpolydactyly (SPD). As seen in Figure 2 in this disorder digits on the hands and feet can be fused together or additional digits may be present. This rare condition was first described in 1916 in an Australian family who faced extreme prejudice and stigma from their community, despite the fact that patients do not suffer from any other physical or mental impairment.

The first thorough genetic investigation into synpolydactyly was in a rural Turkish family that had 182 affected members. Their genomes provided the molecular basis for the study, which located the HOXD13 mutation. In some extreme cases where the 14-alanine gene was expanded male children were also born with hypospadias, a birth defect where the urethra does not open up in the correct location.¹⁴

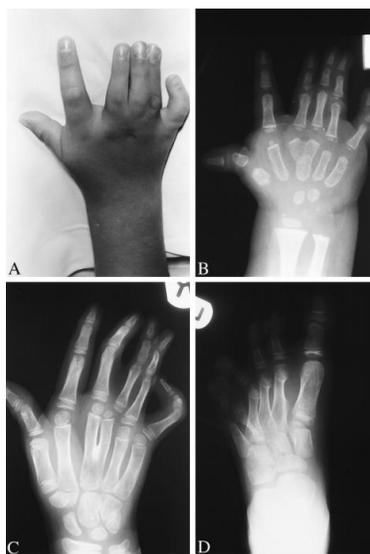


Figure 2: Synpolydactyly (Source: Goodman, Mundlos, et al., 1997)

¹⁴ Grier et al., 159.

Curiously, the known human Hox gene mutations primarily only affect the far distal parts of the hands and/or feet and occasionally the urogenital tract. It is possible that Hox gene mutations affecting other parts of the body have not been fully described and researched yet. Additionally, any other mutations of the human Hox genes so far could have been lethal. Developing embryos with Hox gene mutations affecting other areas could have defects so severe that embryonic death is inevitable.

Hox Genes and Oncogenesis

New research into Hox genes has uncovered that they have a significant role in oncogenesis, the formation of cancer. The protein products of Hox genes act as transcriptional factors that promote carcinogenesis, the initiation of cancer formation, by being upregulated or downregulated in cancer cells. To say more clearly, in cancer cells the quantities of Hox gene protein products are off - either too much or too little is created.¹⁵

While Hox genes have been implicated in dozens of different types of cancer, only colon, breast, and prostate cancer will be directly addressed for clarity and simplicity. In colon cancer the HOXA family of genes is downregulated, while the HOXC family of

¹⁵ Bhatlekar, Fields, Boman. 2014. HOX genes and their role in the development of human cancers. *Journal of Molecular Medicine*. 92:811-823.

genes are slightly upregulated. In this case the overexpression of HOXB7 contributes to tumorigenesis.¹⁶

In breast tissue the HOXA5 gene codes for proteins that induce intentional cell death known as apoptosis in breast cancer cells. The expression of this tumor suppressing protein, p53, is deficient where HOXA5 expression also is. There are HOXA5 binding sites in the p53 promoter, whose expression induces p53 expression in breast cancer.¹⁷ Essentially, the downregulation of HOXA5 leads to much less of the tumor-fighting p53 gene being made. As a result pre-cancerous cells are not eliminated and can continue to grow, and breast cancer is far more likely to develop.

Research with mice as described in Figure 3 has shown that HOXA5 presence is essential for p53 to be effective. HOXA5 homozygous mutants had drastically lower lifespans across the board, regardless of whether the specimen possessed a mutant p53 gene or not. Most HOXA5 homozygous mutant mice died within the first day, which hints that this specific mutation could be considered somewhat lethal. This

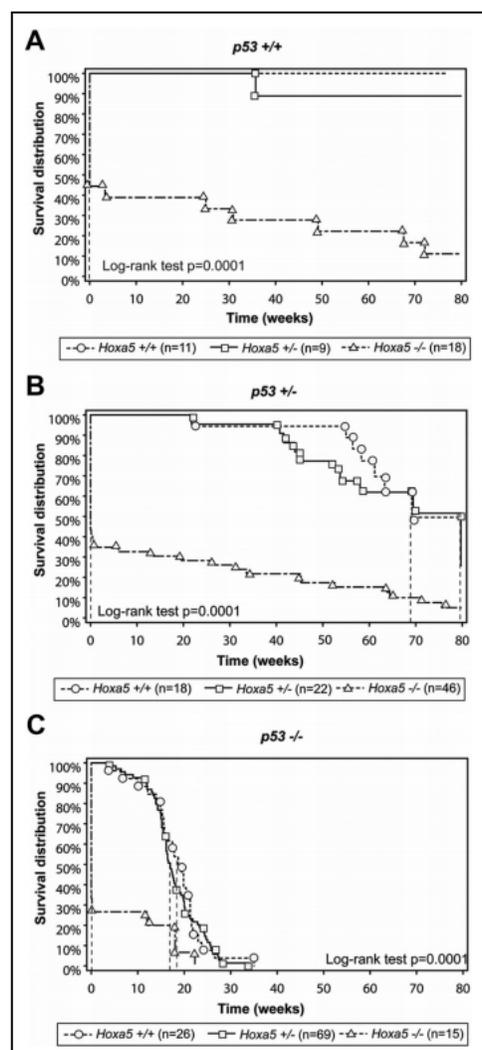


Figure 3: HOXA5 and p53
(Source: Gendronneau et al., 2010)

¹⁶ Bhatlekar et al., 812.

¹⁷ Shah, Sukumar. 2010. The Hox genes and their roles in oncogenesis. Nature Review/Cancer. 10:367.

mechanism of intertwining between HOXA5 and p53 is not specific to only breast cancer. HOXA5 and p53 can be found throughout the body wherever tumors might grow.

Meanwhile, in prostate cancer the HOXC family are overexpressed. HOXC8 specifically has a role in invasiveness and the metastasis, or spread, of prostate tumors. Many other cancers, including but not limited to lung cancer, glioblastoma multiform (GBM) brain tumor, thyroid cancer, and ovarian cancer, have been linked to incorrect Hox gene regulation. Of the 39 human Hox genes, only two (HOXC10 and HOXC12) were not expressed in some kind of tumor. The two most commonly altered Hox genes in tumors are HOXA9 and HOXB13.¹⁸

This knowledge about Hox gene involvement in cancer is useful. Understanding the role of Hox genes in oncogenesis might aid in designing therapies to treat cancer in humans. However, the unresolved question is whether the upregulation and downregulation of Hox genes are causes of carcinogenesis or whether they are simply effects of cancer.

Assuming that the different levels of Hox gene expression contribute to cancer, therapies could theoretically be produced to target those same genes. Being able to reverse upregulation or downregulation and return Hox gene protein products to their normal levels of expression might be able to prevent cancerous cells from growing or

¹⁸ Bhatlekar et al., 813.

multiplying. Of course, this is under the assumption that these Hox genes are the only driving force behind the cancer. In reality this is probably not as simple.

Differentiated-expression of Hox genes could also be a factor in numerous other diseases, and given the time and money further research could investigate whether their expression levels are linked to other disorders. This knowledge could potentially allow for the treatment or prevention of many of the common ailments if it is found that Hox genes play a role in their formation. To speculate, Hox gene dysregulation could be intricately involved in other kinds of congenital limb defects, and understanding how they are expressed could be the key to treating these problems as well.

Even if the upregulation or downregulation of Hox genes are simply effects of carcinogenesis, they can still be useful. If a baseline expression level of the various Hox genes could be determined in different organs or parts of the body, then any change in these levels could serve as a biomarker to screen for cancer in the very early stages.¹⁹ A longitudinal study that is somehow able to monitor Hox gene expression levels in healthy and cancer-risk subjects could determine when this dysregulation occurs. If these changes can be observed before tumors or cancerous cells are detected, then it could be theoretically possible to begin treating cancer before it even begins.

¹⁹ Bhatlekar et al., 821.

Conclusion

Usually when the general public thinks of genes they immediately jump to obvious traits like blue eyes or hair color. There are 20,000 to 25,000 human genes, and most do not code for anything obvious. The vast majority of genes code for far more fundamental parts of the human body. Hox genes are just 39 of thousands that code for the nitty-gritty foundation that humans need to survive. While they may not get as much attention as the “glamor” genes that code for appealing physical features, Hox genes are just as necessary and important.

Any small change in the nucleotides that make up these 39 genes can result in the death of a developing embryo. Even when some individuals are able to survive a Hox gene mutation, it is only with significant impairment. These facts rely just how important Hox genes are. They are a part of the hidden genomic infrastructure without which we would be unable to survive.

Research into Hox genes has opened the door to greater human understanding of genomics, evolution, and cancer. Their colinearity is a novel feature that thus far has been entirely unseen in the genome, and their structure provides enormous insight into the mechanisms of evolution for extremely conserved genes. Some of the rarest human genetic disorders have been traced to specific mutations in Hox genes, and only recently has research made the connection between Hox genes and cancer.

Cancers are incredibly complex and varied, but new research has given great insight into how Hox genes are involved in this process. Utilizing this knowledge with further research is essential to developing ways to possibly treat certain cancers, or at least the very least use Hox gene regulation and expression as a biomarker for cancer screening and prevention.

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