Cancer in Ancient Human Populations: Methods and Practice in Bioarchaeology and Paleopathology

Undergraduate Research Thesis

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Abstract: Despite its prevalence in contemporary public health, research on the paleopathology of cancer is still extremely limited. Successful methods have been employed to identify cancer in human remains which show a very small fraction of the existing archaeological record to contain signs of cancer. This current evidence would indicate that cancer was much rarer in antiquity than it is now, and this would suggest that cancer is a product of modern day environments and lifestyles. However, this conclusion is based upon very narrow research utilizing a methodology that is limited in its reach. Current methods rely solely on gross observation of skeletal material, which fails to account for the wide range of factors that influence the growth and development of carcinomas. This methodology is insufficient in providing a detailed history of the growth and development of cancer in human antiquity. This project aims to determine an interdisciplinary methodology for the study of ancient human cancers, incorporating approaches employed in bioarcheology, epidemiology, and more contemporary cancer genomics.

Introduction

Cancer has dominated discussions of public health throughout the 21st century. According to the spending reports provided by the National Institute of Health, over six billion dollars were used by the organization to fund cancer research in 2018. This spending is in part because the disease occupies such an enormous space in the public imagination. Despite the fact that cancer kills fewer people than heart disease, it is widely perceived to be the most frightening disease with which someone can be diagnosed (Awang et al., 2018). A diagnosis of cancer is often perceived as a death sentence, even in cases of cancers with good prognoses. Contemporary popular culture is flooded with references to cancer, from blockbuster films to

teen romance novels. Organizations promoting cancer research have become so familiar that many of us instantly associate the color pink with breast-cancer. The disease holds an unrivaled presence in contemporary discourse on human health, one that will only grow as the human lifespan gets longer and our understanding of cancer's mechanisms improves.

To understand what makes our relationship to cancer as humans so distinctive, it is important to understand what makes cancer such a unique illness in the first place. When we use the word disease, it is often in reference to infectious conditions, i.e. outside pathogens that put our bodies at risk. These pathogens elicit a fear of exposing ourselves to something that may make us sick. With this comes a sense that we have the power to protect ourselves from infection: a belief that agency over our own bodies gives us the power to avoid becoming sick.

With cancer, the agent of harm comes from within our own bodies. A review of studies on why people fear cancer so much showed that it was the *internal* element of cancer that contributed to fear in many cases (Vrinten et al., 2016). People cited the hidden nature of cancer, its ability to grow undetected within one's own body until it is too late to stop, as a primary fear. Several individuals in the study referred to cancer as a betrayal; the body that they thought they understood had become an enemy (Vrinten et al., 2016). While there are numerous other diseases wherein the body attacks itself (i.e. autoimmune diseases, several genetic diseases), for many people, cancer is understood as the ultimate failure of our bodies to protect ourselves. In part, the weight given to cancer is due to the dominance it has had in public discourse throughout modern history. The discovery and study of autoimmune diseases did not begin until the beginning of the 20th century, while the existence of tumors has been at

the very least accounted for thousands of years (Rifkin et al., 2017). This longevity of knowledge of cancer has allowed it to occupy a great deal of space in our cultural representation of disease, which in turn has further built upon the mythos of cancer as the most nefarious of illnesses.

For all of the gravity cancer holds within global society today, we know precious little about how it manifested in the past. While cancer has been documented throughout human history, its presence in the archaeological record remains minimal. Evidence from literature dates as far back as to ancient Egypt, where medical papyri contain descriptions of how to remove swellings that could very well be what we today consider cancer. Ancient Greek writings referred to swellings and masses as karkinomas, from the Greek word for crab, which early tumors were thought to resemble (David and Zimmerman, 2010). This association of tumors with crabs continues to this day, as the word cancer is also the Latin word for crab. The historical record would indicate that cancer was at the very least common enough to be a topic of importance in medical texts, despite limitations in the physical evidence for its presence.

The disparity between textual evidence and physical evidence for cancer leads us to the central question in discussions of paleo-oncology: to what degree is cancer a product of modern environmental and lifestyle factors? By studying the ancient presence of the disease, we can expand our understanding of how it became such a prominent health crisis in modernity.

Existing Paleo-oncological Evidence

As of now, much of the work towards determining the modernity of cancer has been done through a qualitative analysis of human remains. A study published by anthropologists A. Rosalie David and Michael Zimmerman argues that the current archaeological evidence points towards cancer's modernity, based on a compilation of data collected from both skeletons and mummies (2010). They found there to be an extremely limited quantity of bone metastases in the collections, specifically citing a study on 133 Egyptian skeletons that found no evidence of malignancy, as well as a general rarity of skeletal signs of malignancy in Egyptian remains. From this, they concluded that the scarcity of physical evidence for cancer was a sign that cancer was a rare disease during antiquity due to an absence of modern-day carcinogens (David and Zimmerman, 2010).

There are numerous explanations that explain why cancer might have been less common in the past. The environment has a substantial impact on cancer formation, as seen in estimates that up to half of all cancers can be attributed to preventable causes, such as tobacco and exposure to UV radiation (Halerpin, 2004). And we also know that the industrial revolution brought with it an abundance of carcinogens that are specific to modern lifestyles. Additionally, the modern diet has a significant amount of potential carcinogens that would not have been present in antiquity, such as chemical preservatives.

Another suggested reason as to why evidence of cancer is rare in the field of paleopathology points to the disparity in life expectancy between modern populations and historic ones. According to the World Health Organization, the average world life expectancy today is approximately 72 years, while average life expectancies prior to modern medicine were

unlikely to go above 50 years (Binder et al., 2014). The risk of receiving a cancer diagnosis increases with age. The probability of being diagnosed within ten years increases up to almost 18% at 70 years of age (White et al., 2014).

This would indicate that individuals living in antiquity would likely have died of infectious disease or other causes before they ever developed cancer, or before the cancer became symptomatic. While it is likely that the age of diagnosis has a significant effect on the rate at which cancer appeared in ancient populations, there are still numerous cases of younger people being diagnosed with cancer. We must account for the young as well in order to study the existence of cancer within past populations.

However, there is also a good deal of evidence that puts David and Zimmerman's into question. For one, the authors focus very specifically on studies of skeletal and mummy samples from ancient Egypt and their respective lack of markers for malignancy. This narrow geographic scope does not account for the very real possibility that rates of cancer were different in different parts of the world due to environmental and genetic factors. Secondly, they conclude that the rarity of skeletal signs of cancer is a sign that cancer was a rare disease. This claim may be backed up by the relative absence of data, but it does not account for limitations in the methods we have utilized to identify cancer in human remains to this point. The gross skeletal observation employed in the studies cited by David and Zimmerman is not exhaustive enough to draw conclusions about cancer's modernity. The lack of observable skeletal data does not necessarily correlate to the prevalence of cancer in antiquity. We cannot simply assume that the lack of physical evidence is proof of cancer's rarity when the tools we have utilized to identify cancer in human remains are so limited in scope.

While the data they cite in their study shows that the skeletal symptoms of cancer are only present in a few cases, synthesizing an estimate of the frequency of cancer in ancient populations requires a different methodology than what they employed (Faltas, 2011). Cancer is an exceptionally complex disease, comprised of genetic, environmental, and behavioral factors. In order to gain a clear understanding of how cancer manifested in ancient populations, we must develop new tools for identifying cancer in human remains, drawing from the existing fields of bioarchaeology, epidemiology, and molecular paleopathology.

Environmental Factors in Tumorigenesis

The conclusion of David and Zimmerman, does not take into account possible environmental conditions that could have produced other kinds of carcinogens in antiquity. For example, Michaela Binder highlights the indoor ovens that were common in ancient settlements, arguing that the smoke from these fires, when trapped in a hut without windows, significantly increases risk of lung cancer (Binder et al., 2014). In order to determine whether cancer is a modern disease, we would first need to study ancient environments and living conditions in order to account for potential risk factors for cancer.

There is currently a lack of substantial research on what types of carcinogens might have existed in the past. In the present day, carcinogens such as tobacco and air pollution are responsible for the majority of cancers, but this tells us very little about how past environmental and lifestyle factors may have contributed to the development of cancer (David and Zimmerman, 2010). The genetic framework that allows for cancer growth may be

consistent, but the external elements that effect the mutation rates of these genes are both

spatially and temporally specific.

Although very little concrete research has been done to identify past carcinogens, we

have a vast knowledge of contemporary common cancer-causing agents. The International

Agency for Research on Cancer (IARC) has released a monograph listing hundreds of confirmed

and suspected carcinogens (2020). We can trace how these agents may have affected past

populations in a multitude of ways. Diets, especially those rich in red meats, are seen as a

prominent factor in the development of several cancers, namely those of the stomach and

colon. Meat consumption, of course, is not exclusive to the 21st century, making it an

identifiable carcinogen to which past populations were certainly exposed. With stable isotope

analysis we have the tools to reconstruct past diets and see what types of organisms were

being consumed. When this technology was used in a study of a colon tumor found in the

mummy of King Ferrante from Renaissance Italy, stable isotope analysis showed a relatively

high δN value, indicating that the king had a meat-based diet (Ottini et al., 2010). This further

validates the researchers' diagnosis of colorectal cancer in the mummy, as these cancers are

considered to be frequently tied to extreme meat-based diets.

Carcinogens occurring in the natural environment would have also played a role in

cancer development in the past. Although the most prominent carcinogens today are man-

made, there remain numerous naturally occurring examples that existed millennia ago.

Asbestos, a crystalline compound used for insulation, is seen as one of the primary causes of

mesothelioma, a cancer of the mesothelium of internal organs. This compound has been

harnessed by humans for thousands of years for its inflammability and ability to strengthen

textiles. Archaeologists are able to find traces of asbestos using x-ray spectroscopy, and have identified it in several historic cases, providing another example of a carcinogen to which past populations would have certainly been exposed (Kakouilli et al., 2013).

The IARC monograph also lists several pathogenic factors that are associated with carcinogenesis, including the human papillomavirus (HPV) and Epstein-Barr virus (2020). Certain pathogens such as these have the potential to damage DNA, causing normal cells to become cancerous—particularly if left untreated. Pathogenic sources of cancer provide yet another point of study when attempting to determine what sorts of carcinogens might have existed in past environments (Rifkin et al., 2017). HPV, for example, is the most prominent cause of cervical cancer in humans (Chen et al., 2018). Researchers traced the evolution of the type most associated with cervical cancer (HPV), finding that it diverged at roughly the same period of time anatomically modern humans began to diverge from other archaic hominins (Chen et al., 2018). From this research, we can see that the oncogenic potential of HPV has been associated with human beings for roughly as long as modern humans have existed.

While we can be certain that modern environmental carcinogens have exacerbated our susceptibility to cancer in the past few centuries, there is clear evidence that plenty of oncogenic factors have been present throughout human history. Cancer arises from an intricate entwinement between our bodies' genetic codes and the environments around us. Studies of bioarchaeological remains showing signs of metastatic cancer must be done in conjunction with a study of that individual's environment. This gives us a way to broaden our understanding of the disease as a whole, by opening up its multifaceted origins and helping us trace its roots throughout human history.

Oncology Background

Cancer is defined as a pathology that is caused by unregulated cell division in the body. Abnormal cell division can result in a growth of tissue known as a neoplasm. Neoplasms can be benign or malignant, meaning they can spread, or metastasize, to other tissue. When a neoplasm is malignant, it becomes pathological and is considered a cancer. There are hundreds of diseases classified as cancers, which are generally distinguished from one another based on the part of the body from which the neoplasm originated. This first neoplasm is referred to as the primary site, while any other locations to which it may have spread would be considered secondary sites.

Cancers are also divided into several groups depending on what type of tissue the neoplasm developed in, with the major ones being carcinoma, sarcoma, melanomas, lymphomas, and leukemias. Carcinomas are the most common form of cancer and originate in the epithelial cells that line organs and blood vessels. Sarcomas originate in connective tissues such as bone and fat. They are much rarer than carcinomas and also effect a wider age range, with several bone sarcomas being more common in children than adults (Rivera-Valentin, 2015). Melanomas occur in the melanocyte cells of the skin, which provide the pigment for one's skin. They are distinct from other skin cancers, which are carcinomas occurring in the epithelial cells of the skin. Lymphomas and leukemias are sometimes referred to as 'liquid tumors' due to the fact that they exist in a fluid form in the blood stream, rather than as solid masses in the body. Lymphomas originate in the white blood cells, while leukemias develop in the bone marrow. The variation in causes, symptoms, and prognoses in these different cancers

is what makes the disease so difficult to study, both in modern medicine and especially in the archaeological record.

One major limitation in the identification of cancer in human remains is the type of human remains that archaeologists have available to study. Only in mummified remains is the soft tissue preserved in the archeological record, and this requires very specific conditions that are limited to certain environments or cultural practices. The majority of what we have available comes in the form of skeletal and dental material. This means that the cancers that will be visible in human remains are primarily going to be bone cancers or cancers that spread to the bone as a secondary location.

In 2019, primary bone cancers represented only 0.2% of cancers in the United States (NIH). Unless the rate of these cancers has significantly reduced in modern times, we can extrapolate that they were also much rarer throughout ancient history, explaining their general absence from the archaeological record. Despite the general rarity of primary bone sarcomas, it is not uncommon for carcinomas to metastasize to the human skeleton as a secondary location. This metastasis is the most common in carcinomas of the prostate, breast, lung, and thyroid (Ortner, 1981). Bone has been shown to be the third most common location that cancer metastasizes to, and such metastases are seen in up to 75% of advanced breast and prostate cancers (Macedo, 2017). Both instances of cancer metastasizing to the bone and of primary tumors developing in the bone can leave behind physical signs of pathology in the skeletons that can be used to identify the presence of neoplastic disease. While the studies cited by David and Zimmerman indicated a scarcity of these markers in ancient Egyptian remains, they have been utilized successfully in several other populations.

Methods for Documenting Cancer in Human Remains

Bioarchaeology

Despite David and Zimmerman's evidence that neoplastic disease is rare in the archaeological record, we are confident that cancer has existed for millions of years, predating anatomically modern humans. The earliest known malignant hominin cancer was identified in an Early Pleistocene hominin from South Africa (Odes et al., 2016). Select other studies have located prehistoric examples of neoplastic disease in archaic humans and early anatomically modern humans (Rifkin et al., 2017). There are several existing qualitative analysis techniques that have been used successfully to identify cancers such as these in human skeletal remains. Like many pathological conditions, certain cancers can leave behind skeletal evidence. In addition to primary bone cancers or metastatic cancers which directly impact the skeleton, soft tissue tumors can cause alteration in bones with which they come in contact. This type of gross analysis is the primary method that researchers have used to identify cancers in the archaeological record. As we have seen in David and Zimmerman's study, these methods have yielded very limited positive identifications of cancers.

Human bone is constantly remodeling itself in order to maintain homeostasis, meaning bone is reabsorbed at a rate equal to bone deposition in most circumstances (Kolb et al., 2019). When a neoplasm spreads to human bone it leads to a stoppage in this careful regulation, creating osteolytic or osteoblastic lesions on the bone. The bone response to the tumor depends on the type and origin of the tumor. Generally, osteolytic effects make out about 75% of bone response in metastatic cancers (Binder et al., 2014). Tumors generally metastasize to

the bone marrow of the skeleton via the blood stream, with the primary bones effected being located around the axial skeleton and the proximal epiphyses of the long bones. The spine is the most common bone seen with signs of tumor in autopsies (Ortner, 1981). Osteolytic lesions form when osteoblast cells are unable to deposit bone at the same rate in which the bone is being reabsorbed. This disrupts the normal remodeling process of the bone, leading to reduced bone density and a high risk for fractures. Bone loss begins in the cancellous bone, though extremely advanced stage cancers can result in destruction of the cortical bone (Binder et al., 2014).

Osteoblastic responses, while less common than osteolytic ones, are much easier to diagnose due to their distinctive appearance. These responses are identified by deposits of bone in areas where bone does not normally develop. They are usually seen in less aggressive, slower growing cancers, namely those of the prostate (Macedo et al., 2017). In some cases, both osteolytic and osteoblastic lesions will be produced in the bone.

The challenge that arises with this type of physical analysis of skeletal material is that it is difficult to make a conclusive diagnosis. Osteolytic lesions due to neoplasms in ancient remains are not always distinguishable from other types of lesions that can form in the bone. These non-neoplastic lesions can result from infections, trauma, and developmental disorders (Kirkpatrick et al., 2018). In addition to these sources of lesions, standard taphonomic damage to the bone can be mistaken for metastatic lesions, and vice versa. When working with excavated human remains, it is expected that the bone would be affected by taphonomic processes such as weathering and interaction with other animals. Because of the confusion that can result from the similarities of these lesions, researchers have struggled to prove the

presence of cancer beyond reasonable doubt in ancient remains. Therefore, macroscopic analysis with the naked eye is an insufficient method for the identification of cancers in human remains.

A study in the *International Journal of Paleopathology* proposed a methodology for distinguishing neoplastic lesions from other types of modification to the bone (Ragsdale et al., 2017). The study establishes *three* parameters that are essential to diagnosing a lesion: margins, periosteal reactions, and matrix patterns. Analysis of these parameters requires that radiological imaging is performed on the remains, as it will allow for the viewing of all lesions in the bone, even those that are not visible macroscopically.

The margins of a lesion are the boundaries in between healthy and diseased bone. The appearance of these margins can be used to distinguish neoplastic and non-neoplastic lesions, as well as classify certain types of neoplasms. The margins are classified into three major groups. Type I margins are well defined and localized lesions. They can either have sclerosis (bone hardening) around the borders or have defined borders with no sclerosis. The sclerosis appears in radiography as a bony shell that defines the margin, and it is associated with bone cysts and benign tumors. Faster growing tumors such as an osteoclastoma would not have sclerosis surrounding the margin, as the tumor will expand before the bone can harden. Type II margins are more indicative of metastatic carcinomas, and appear as smaller, overlapping lesions. However, these margins can also appear in fungal infections, making definite diagnosis from them difficult. Type III margins are fairly similar to Type II, and diagnose for relatively similar neoplasms (Ragsdale et al., 2017).

Periosteal reactions are the formations of bone that occur on the very outer layer of bone, known as the periosteum. These reactions can either result in positive or negative additions of bone material. Negative additions result in the deletion of existing cortical bone, while positive additions add to the cortical bone and do not form margins in the bone. Malignant cancers tend to show positive periosteal reactions, particularly highly lamellated reactions. While reactions of a single lamella being formed are indicators of benign tumors, lamella will take on an onion skin form in the case of sarcomas and a parallel spiculated form in malignant carcinomas. Some tumors create a matrix, the pattern of which can be analyzed through radiography to diagnose the disease. Matrices of osteosarcomas tend to have poorly defined margins and a semi-solid structure. Using radiography and a careful analysis of the features of bone lesions, we can narrow down the specific pathology that caused them, leading to more accurate diagnoses.

Through the techniques outlined above, we can better diagnose tumors in ancient human remains. We can see these techniques put into use in a study on a skeleton found in Ancient Nubia, in the Amarna West site of modern-day Sudan. The skeleton found had numerous lesions located in the vertebrae, sternum, clavicles, pelvis, and the proximal humeral and femoral heads (Binder et al., 2014). All of these are the most common secondary locations for carcinomas to metastasize. This would indicate that the individual suffered from some sort of metastatic carcinoma, but more evidence is needed in order to make a diagnosis. Thus, researchers performed radiographic imaging in order to more clearly see the margins of the lesions.

From these radiographic images, the researchers narrowed the potential diagnoses down to a few options. The most likely non-neoplastic option was determined to be mycosis, a fungal infection that can produce lesions in the bone. However mycosis generally produces lesions throughout the body, and not solely in the axial skeleton. The most likely cancers that could produce the osteolytic lesions found would be a malignant carcinoma or multiple myeloma, which is a cancer of the plasma cells. The lesions of multiple myeloma are distinguishable from carcinomas in the appearance of their margins. Multiple myeloma produces very rounded, well-defined lesions, as seen in the Type I margins defined by Ragsdale. However, the lesions found on the Nubian burial contained margins closed to Type II, as they were irregular in size and had poorly defined shape. The lesions also showed some new bone formation in the pelvis and vertebrae, which does not occur in multiple myeloma. Finally, the study showed that the lesions on the burial were distinct from taphonomic damage. The fact that the lesions were specific to the axial skeleton was a good indicator that they were not taphonomic in origin, as was the formation of new bone, which would not occur postmortem. This led researchers to conclude that the individual most likely suffered from a metastatic carcinoma. It was not possible to determine the primary site of the cancer, due to the absence of soft tissue. However, they were able to indicate the most statistically likely sources based on which cancers most commonly metastasize to the bone and produce osteolytic effects. These would include cancers of the lung, breast, thyroid, and liver.

This study provides further physical evidence that cancer did exist in antiquity, and that part of the reason the disease has been so difficult to identify is due to the similarities between paleopathological evidence for cancer and other diseases. Even if a confident diagnosis of a

neoplastic disease can be made, the methodology for narrowing it further is limited (Kirkpatrick et al., 2018). Without consistent radiographic imaging, it is very easy for researchers to mistake signs of neoplasm for taphonomy or infection of the bone. This is a very likely explanation for the current lack of archaeological evidence for the antiquity of cancer, indicating that we must expand our methods beyond observation of skeletal material with the naked eye.

Epidemiology

In a paper published in the *International Journal of Osteoarchaeology,* Zuckerman, Harper, and Armelagos argue that a failure to adapt epidemiological methods in the study of ancient cancers has lead us to make incorrect assumptions about the degree to which cancer impacted human populations in the past (Zuckerman et al., 2016). Namely, they call out the study by Zimmerman and David as insufficiently looking at how the frequency of skeletons showing metastatic lesions compared to what we would expect in a modern population (Zuckerman et al., 2016). A 2005 study in *Oncology Reports* attempted to calculate the number of occurrences of neoplastic disease in a sample population based on skeletal analysis. The study looked at two populations, a series of Egyptian burials dating from the early dynastic period to the New Kingdom and a German cemetary containing bodies from roughly the 15th through 19th centuries (Nerlich et al., 2005). The number of instances of neoplastic disease found in these burials was used to estimate an overall frequency for cancer in the respective populations. When this frequency was compared to that of a modern day English burial, they were found to be not statistically different for both the Egyptian and German populations (Nerlich et al., 2005). This indicates that the frequency of bone metastasis in these two

populations was not significantly lower than what we see in modern populations. Of course, this study only provided proof of this for the populations examined. Nevertheless, the results raise significant questions about the assumption that David and Zimmerman drew about the prevalence of cancer as a modern phenomenon.

The Nerlich study employs the methodology suggested by Tony Waldron in 1996 for estimating the expected frequency of cancers that would metastasize to the bone in past populations (Nerlich et al., 2005). Waldron's methodology utilizes two data sets on contemporary cancer deaths, one from 1901-1905 and one from 1993, both coming from the England's Registrar General. Understanding that different primary neoplasms have different frequencies of metastasizing to the skeleton, Waldron calculated the frequency with which we would expect to find bone metastases in each site of cancer reported by the Registrar General by multiplying the known percentage of tumors of that type that metastasize to bone (i.e. 75% of prostate cancers) by the percentage of total deaths that were due to cancer of that site.

A summation of these frequencies gives us an estimate of how many skeletal metastases we would expect to find in a given population (Waldron, 1996). Waldron's work relies on dated statistics, particularly those from 1901-1905, but the calculations he used can easily be applied in conjunction with more contemporary death records in order to update the expected frequency of bone metastases for the 21st century. With Waldron's methodology, we can consistently apply epidemiological techniques to the study of paleo-oncology. This would allow us to go beyond simply identifying signs of metastasis in human skeletal remains and gain a greater understanding of the degree to which cancer effected specific populations in the ancient world.

Molecular Paleopathology

The inherent limitations of qualitative skeletal analysis are that it requires the presence

of a visible neoplasm in the skeletal tissue in order to be effective. This means that any estimate

for the prevalence of cancer in antiquity based solely upon skeletal observation will necessarily

provide an incomplete picture due to the lack of soft tissue evidence. It will not allow us to

specifically identify the primary tumor site in cases of metastasis to the skeleton, only acting to

confirm that the cancer has in fact spread to the skeleton. This makes it more difficult for us to

understand which cancers were prevalent in different time periods. Ancient DNA (aDNA)

analysis offers the potential to more specifically identify and categorize the neoplasms

observed in both skeletal tissue and in the soft tissue of mummies.

Our increased understanding of the genetic factors behind cancer has allowed scientists

to identify many more specific triggers for oncogenesis. The human genome is extraordinarily

complex, comprised of billions of base-pairs. However, despite the scale of human genetic

material, only roughly 1% actively code for proteins, with the remaining non-coding DNA

performing a vast set of functions that are still being uncovered by geneticists (Zhao, 2012).

With whole-genome sequencing technologies, we have been able to narrow the number

of genes that act as drivers for cancer to roughly 140 genes (Kim, 2015). These genes are

categorized as either *tumor suppressors* or *oncogenes.* Tumor suppressors, when unaffected by

mutation, are responsible for controlling the division of cells. Some tumor suppressors are

referred to as 'gatekeeper' genes, and directly act to prevent cell division. The most dangerous

mutations in these genes are those that those that directly disable the protein that they code

for, thus rendering their ability to maintain cell division obsolete. On the other hand, 'caretaker' genes work to moderate the DNA replication process by identifying and repairing coding errors (Deininger, 1999). A mutation which deactivates the function of these caretaker genes does not directly lead to the uncontrolled growth of cells, like in the case of the gatekeeper gene, but rather allows for more coding errors to build up in the genome.

Oncogenes simply refer to any gene that has the potential to cause cancer when their protein product is changed by mutation. Unlike tumor suppressors, the types of mutations that cause these genes to become oncogenic are not those that do not deactivate the gene, but rather those that alter the amino-acid structure of the protein so that its function changes. This leads to severe cellular ramifications, seen either in the overexpression of these genes and an overabundance of their protein products or in a lack of control over the activation of these genes, causing the unregulated cell growth that leads to tumorigenesis.

Massive breakthroughs in gene sequencing have led to novel treatments for cancers focused on the specific genetic factors associated with these cancers. The ability to sequence the genomes of cancer cells opens up the landscape of treatment beyond what previously was possible. Targeted therapy acts on specific molecules associated with tumor growth, providing a much more specified treatment than chemotherapy and radiation therapy (Kim, 2015). Many of these tools used in targeted therapy for cancer patients today can prove beneficial for the study of how cancer existed in the past, as they allow us to identify the biomolecular roots that allow for the development of cancer. In this section, we will look at the most common mutations that act as markers for the presence of cancer and show how these same markers can be found in ancient DNA.

There have been very few attempts to directly identify oncogenic mutations in ancient DNA. As of 2017, there were only two extensive studies on the genomes of ancient tumor samples. One successful identification was a mutation of the KRAS gene in the mummy of King Ferrante of Aragon in 2010 (Ottini et al.). The researchers had identified what appeared to be a tumor in the pelvic area of the mummy and performed aDNA extraction on the tumor sample. Polymerase chain reaction amplification, a way of targeting specific DNA sequences and duplicating them so that they can be visualized, was then performed specifically looking for the KRAS gene.

The KRAS protein, when activated, sends biochemical factors that induce cell division. KRAS mutations are found in roughly 20% of the samples accounted for in the *Catalogue of Somatic Mutations in Cancer* (COSMIC) database (Forbes et al., 2008). Mutations in KRAS can lead to its activation, triggering cell division without the molecular control that mediates the activity of wild-type KRAS. King Ferrante was found to have a base pair substitution of guanine to arginine in its twelfth codon, which would lead to a missense mutation (a mutation that changes the amino acid product that the base pair codes for), the most common mutation type to activate oncogenes. COSMIC data supports the hypothesis that it is this mutation that lead to the cancer's growth, showing that this mutation is most frequent in cancers of the large intestine, which is in line with the intestinal region where the tumor was found (Ottini et al., 2010). Earlier, we discussed the case of King Ferrante in the context of dietary reconstruction, as he was found to have consumed a predominantly meat-based diet. This finding would offer a possible genetic explanation for the origin of the king's cancer. The KRAS oncogene has been shown to be vulnerable in heavily meat-based diets (Naguib et al., 2010). This allowed

researchers to draw a direct connection between the environmental and lifestyle roots of the cancer (i.e. diet) and the genetic factors that directly lead to oncogenesis.

Another study sampled the soft tissue of the colon of three 18th-century Hungarian mummies. The researchers performed DNA extraction and were able to amplify the APC gene (Feldman et al., 2015). This gene is a tumor suppressor that, when deactivated by mutation, leads to a high risk for colon cancer, in particular. Of the three genomes sequenced, one had a missense mutation in the APC gene. This mutation, a substitution of guanine to cytosine in the 1,317th codon, is likewise mostly seen in cancers of the lung and stomach. This study is notable in that the researchers were not sampling directly from known cancer tissue. The presence of the APC mutation was found in what appeared to be normal mummified colon tissue, indicating that the mummy was predisposed to colon cancer due to the mutation (Feldman et al., 2015). Both in this case and that of King Ferrante, ancient DNA analysis proved successful in amplifying cancer-related genes and identifying mutations.

Unfortunately, these studies rely on the presence of soft tissue in mummified specimens. In order to gain a clearer sense of the incidence rate of cancer in antiquity, we will need to be able to draw from skeletal evidence, as the vast majority of human remains do not have soft tissue that can be sampled. In skeletal remains, we do not have access to actual tumor tissue, making it much more difficult to sequence tumor DNA. However, as stated earlier, human bone is one of the most common sites of metastasis. Thus, we can expect that if cancer was indeed prevalent in antiquity, there will be a noticeable quantity of human skeletons that contain metastatic lesions. Since we already know the most likely parts of the skeleton that metastasis occurs in (the axial skeleton, namely vertebrae, and the proximal ends of the long

bones) we can use these sites for our initial samples of skeletal material for aDNA analysis. This allows us to develop a consistent protocol for sampling skeletal material for the purpose of DNA amplification, even in cases where skeletal signs of metastases are not observed.

Tumor DNA was successfully extracted and amplified from a roughly 2,500 year old skeleton from Serbia containing numerous osteolytic and osteoblastic lesions in a 2007 study at the University of Goettingen (Schlott et al.). The researchers took samples from the femur of the skeleton, using this for DNA isolation and were able to amplify the sequence of the prominent tumor suppressor p53. Although researchers found no evidence of mutation in the p53 gene, they did prove that ancient DNA solely obtained from skeletal material can be used to sequence oncogenes and tumor suppressors. This is significant to future studies of paleo-oncology as it shows us that it is possible to perform DNA extraction and identify genes without access to soft tissue.

The Schlott study has massive ramifications in part because the gene identified, p53, is one of the most commonly mutated genes in human cancers, alongside PIK3CA (Kim, 2015). P53 acts as a caretaker tumor suppressor, playing an active role in the location and repair of errors that occur in DNA during cell division. If p53 is damaged by mutation, it opens up the risk of future oncogenic mutations building up in the genome. P53 is mutated in roughly 42% of all cancers, making it a very valuable target for PCR in aDNA analysis (Kim, 2015). PIK3CA mutations, though less frequent than P53, are seen in 18% of cancers (Kim, 2015). As an oncogene, PIK3CA is an attractive target for drug inhibition with the goal of stopping its activity in cancer patients to stop tumor growth.

The degree to which these specific genes are linked to the development of cancers has led to speculation on their role in human evolution. Oncogenes and tumor suppressor genes are among the oldest in the human genome and can be traced back to the first instances of multicellular life upwards of 750 million years ago (Makashov et al. 2019). Why would these genes so linked to a deadly human disease be preserved by evolutionary forces that we might expect to select against? Research on the evolution of these cancer-related genes shows that they are in fact more prone to negative selection, meaning selection against damaging alleles, than genes that are not involved with tumorigenesis (Thomas et al., 2003). What this suggests is that it is the essential function these genes play in overall control and functionality of the cell division processes that causes them to be so highly preserved. Although they undergo strong selection against deleterious alleles, mutations that do arise in these genes are among the most catastrophic for our bodies. We can conclude from this that the genomes we sequence from roughly any point in human history will contain very similar versions of these cancer-related genes. This allows us to directly compare the way these genes appear in ancient DNA to contemporary template copies and identify any mutations that are present in the historical samples.

Discussion

As we have seen, contrary to suggestions that cancer is a product of our present-day environmental factors, the development of cancers is a far more complex process than simply exposure to modern carcinogens. The molecular factors involved in tumorigenesis are deeply

rooted in the evolution of multicellular organisms, and humans have been exposed to naturally occurring cancer-causing agents for all of history.

The existing evidence for cancer as a disease of antiquity is slim, but this is in part due to the adolescence of the field of paleo-oncology itself. As a relatively new science, paleo-oncologists are still developing a consistent methodology for the analysis of human remains. The standardization of these diagnostic tools will also help researchers in developing a systematic record of ancient cancers. The Cancer Research in Ancient Bodies (CRAB) database developed by the Paleo-Oncology Research Organization is one such attempt to create a collaborative documentation of paleo-oncological studies (Hunt et al., 2018). The database has grown to include nearly 300 different examples of neoplastic disease in the archaeological record, encompassing a large geographic and temporal span.

As we continue to collect this data, paleo-oncologists will be able to develop more accurate and consistent methodology for identifying cancers. We will also be able to compare frequencies of different diseases over time and space in an attempt to better understand how the disease has evolved throughout our history. The more we understand about how cancer has changed with our lifestyles and environment, the more we will understand the disease as a whole.

Humanity's relationship with cancer has certainly evolved as it has grown to be a direct cause of death due to our increased lifespan. The development of a consistent methodology for studying paleo-oncology will benefit paleo pathologists hoping to study the histories of ancient disease, but it will also give us a greater understanding of a disease that has been a part of our life history for the entirety of our time on earth.

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