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Molecular paleopathology and paleo-oncology–State of the art, potentials, limitations and perspectives

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ABSTRACT

This paper reviews the current knowledge on molecular paleopathology with respect to oncological information. This covers both the information on the protein level (proteome) as well as the gene level (genome) and includes data on carcinogenic factors – such as molecular evidence for oncogenic viral infections.

Currently, relatively little data is available for neoplastic disease in paleopathology. Likewise, few studies describe the biochemical or immunohistochemical analysis of tumors – a tool to potentially classify the tumor type and the underlying primary tumor in metastases. On the gene level, two studies described distinct molecular mutations in either a tumor-driving oncogene or a tumor suppressor gene, both being excellent examples for paleo-oncological studies.

The paucity of historic tumor material – particularly when only osseous remains are available – represents the most hindering factor for molecular paleo-oncology. This can only be overcome in future by both the thorough investigation of mummified archaeological biomaterial and the improvement of analytical assays in order to trace even minute amounts of tumor material in osseous lesions.

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1. Introduction

Cancer is one of the leading causes of death in modern Western populations, currently ranking second place behind cardiovascular diseases. The prevalence of cancer cases has been increasing, and nearly doubling over the last thirty years (Boyle and Levin, 2008). Recently, an apparent increase in the cancer prevalence through ancient, historic, and modern populations has been debated (David and Zimmerman, 2010). There is still an open debate regarding whether this is due to an increase in environmental and lifestyle related factors, such as pollution, smoking habits and/or the use of harmful dietary constituents (David and Zimmerman, 2010), or merely reflects the dramatic increase in life expectancy in the last 100 years due to successful infection prevention and medical treatment (Waldron, 1996; Nerlich et al., 2006; Nerlich and Bachmeier, 2007). This apparent increase in cancer prevalence may also be a reflection of the improvement in the detection and identification of cancers, and an increase in records kept on cancer cases as taboos have subsided. Paleo-oncological evidence is also prone to destruc-

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tion through taphonomic processes, resulting in limited physical evidence for cancer in the archaeological record.

During the last few decades, an increasing number of cancer cases have been described in the paleopathological literature, most of which is manifested in skeletal human remains. In 1976, Strouhal described fewer than 20 cases with obvious cancer diagnoses in the first compilation of presumed cancer cases from ancient Egypt (Strouhal, 1976), while a much more recent analysis of ancient Egyptian findings provided 60 cases (Giuffra et al., 2004). Unfortunately, very limited demographic data are available on the investigated ancient Egyptian populations, so the cancer prevalence in distinct populations remains largely unknown. The increase in detected cases may be due to the growing number of field (and museum) investigations of human remains, but may also be the result of improved diagnostic tools and a better awareness of paleopathological lesions diagnostic, or at least suggestive, of cancer.

Along with an enhanced scientific interest in paleo-oncology, novel techniques in the analysis and investigation of ancient remains, such as X-rays and CT scans, have been used more frequently. Refined invasive techniques including histology, biochemistry/immunohistochemistry, and – more recently – molecular biology may have also contributed to this increase in case numbers. In this respect, paleo-oncology strongly profits from the significant scientific progress in modern cancer research – and may, in turn, offer important information (e.g. on the "evolution" of certain features of carcinogenesis) for modern oncology.

This paper will not only present a "state of the art" compilation of relevant observations on molecular paleo-oncology, but is also devoted to a critical discussion on limitations, perspectives and applications of molecular studies in ancient tumor material. Focus is given to the two major "levels of action", the tumor proteome and the tumor genome, which are closely associated with each other but provide very different information for paleo-oncological research.

2. Tumor proteomics

Cancer is not only a very complex disease; it is a multitude of various diseases that affect different organs and tissues with the final common outcome of local tissue/organ destruction and/or systemic deleterious spread (metastatic disease) (Kerr et al., 2016). Cancers are genetic diseases. When affected cells lose their control over distinct cell functions, tumor cells show abnormal growth behaviour. Mostly, this is reflected by altered communications between tumor cells and neighbouring "normal" cells (e.g. stromal or immune cells). The extent of this loss of control may be monitored by the ability of tumor cells to retain certain cellular functions of their "original" tissue function, a condition termed "differentiation", which is reflected by the ability to express certain proteins (such as receptors, hormones, etc.). This ability may be lost in the malignant state, a condition termed "dedifferentiation" (WHO, 2015).

Although tumor cells gradually lose their physiological function with rising dedifferentiation, better differentiated tumors can be identified by those protein markers that resemble or mimic "normal" tissues and cells (Kerr et al., 2016). For example, breast cancer cells (like the cells from other hormone dependent female tumors from the uterus and ovary) mostly express receptors for sexual hormones, the estrogen and/or the progesterone receptors, that are physiologically relevant for the normal breast epithelium cells to react on hormonal stimuli, e.g. during lactation (Kalia, 2015; Falco et al., 2016). The expression of estrogen or progesterone receptors may therefore be used to identify breast cancer cells (and/or tumor cells from other hormone-sensitive tumors of the uterus or ovary). This means, in turn, that the biochemical or immunohistochemical identification of hormone receptors in paleopathological material may support the diagnosis of breast, uterine, or ovarian cancer.

Table 1
Examples of biochemical/immunohistochemical tumor markers for the identification of tumor cell line differentiation in paleopathology.

Marker	Tumor cell type
Cytokeratins (various)	Epithelial (vs. mesenchymal) tumor
Estrogen/progesterone receptor	Breast, ovary, uterus carcinoma
Prostate specific antigen (PSA), prostate specific alkaline phosphatase (PSAP)	Prostate carcinoma
Melanoma markers (e.g. HMB, Melan A etc.)	Malignant melanoma
Thyreoglobulin	Thyroid cancer
Liver cell markers (e.g. OCH-1 etc.)	Liver cell carcinoma
CA 19-9	Pancreatic/bile duct cancer
CDX 2-88, cytokeratin-20	Upper gastrointestinal cancer
Various CD-antigens	Lymphoma

Similar expression patterns do exist for other cells with specific organ differentiation which may still be displayed by tumor cells. For example, prostate cancer can be specifically identified by the expression of the prostate specific antigen (PSA) or the prostate specific alkaline phosphatase (PSAP) (Falco et al., 2016). Another example is malignant melanoma, a malignant tumor of the pigment forming cells of the skin (Foth et al., 2016). While the ability to form the typical melanin pigment may have already been lost in the tumor cells, typical pre-cursor proteins, e.g. the HMB-45-protein, may still be expressed by melanoma cells so that they can unambiguously be identified even in non-pigmented tumor lesions. Several more markers are listed in Table 1.

3. Tumor proteomics in paleopathology

Despite the clear diagnostic power of biochemical and/or immunohistochemical tumor type identification, it has rarely been used in paleopathology. Nevertheless, Schultz et al. (2007) identified a 2700 year old case of biochemically proven prostate carcinoma in the skeleton of a Scythian King from Arzhan (Siberia, Russia). This case is not only one of the very rare cases with such a specific diagnostic application, it remains the oldest case of proven prostate cancer in history. It is also very remarkable as the positive tests were done on skeletal tissue, i.e. the osseous metastases of the prostate cancer. The report by Schultz et al. (2007) clearly proves that even the small amounts of tumor tissue entrapped in osteoblastic bone metastases are sufficient for successful detection and identification.

This is also shown in a much more recent case (Nerlich, unpublished data) where we detected the residues of prostate cancer in a macroscopically presumed metastatic lesion of a rib in a Saxonian crypt mummy through the immunodetection of the prostate-specific antigen (PSA) (Fig. 1). Similarly, through the immunohistochemical identification of (pan-)cytokeratins in tumor tissue, Ottini et al. (2011) were also able to confirm a macroscopically suspected diagnosis of colorectal carcinoma in the very well-preserved mummy of an Italian Renaissance king, King Ferrante I of Aragon (1431–1494).

Further molecular information with reference to paleo-oncology may be revealed through complex biochemical analyses. As an example for the potential of those studies, Bona et al. (2014) showed a "tumor-typical" pattern of various proteins (as studied by mass spectrometry) in a case of a 2000-year-old osteogenic sarcoma. The study identified tumor biomarkers, such as annexin A10, heat shock protein (HSP-ß6), transferrin, BCL-like protein, and others. Those biomarkers provide evidence for a (general) "tumor phenotype" without allowing distinction between different tumor

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