Molecular biomarkers in skull base chordoma

Citation for published version (APA):

Document status and date:
Published: 01/01/2020

DOI:
10.26481/dis.20200909yy

Document Version:
Publisher's PDF, also known as Version of record

Please check the document version of this publication:
• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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Summary

In this thesis, we present our focus of biomarker research in skull base chordomas. Biomarkers are defined as biological substances or characteristics, either qualitative or quantitative, that outlines a certain (patho)physiological state and is often used as a surrogate to guide clinicians to indicate disease severity, treatment response and prognosis. Here we summarize the most important findings of the chapters.

The first manuscript in chapter 2, presents a summary of the entity called chordoma. As many important historical and recent findings shaped our current thoughts and ideas of chordoma, we accentuate certain discoveries that are a mainstay in how we view the pathophysiological state in chordomas today. First, we admire the human embryology by describing the formation of the notochord, an embryonic elongated rod of cells that (amongst others) guides the formation of the vertebral axis and skull base by secretion of important signaling factors, the vertebral axis and the skull base. To illustrate the beliefs that form our current concept of chordomagenesis, the etiology of chordomas, we highlight the historical development of several paradigms and what we now consider the physiological foundation. By displaying a hypothesized model of these mechanisms, we inform the reader of the possible scenarios for chordomas to arise. The molecular groundwork responsible for tumor development and subsequent tumor behavior, has been very diverse over the past two decades, and primarily performed by single center studies. Here, we discuss (epi-)genetic findings, with the predominantly observation of chromosomal instability in chordoma cells and a proposed single crisis event that is advocated to precede it, called chromothripsis. This genetic backbone of the disease is very heterogeneous, possibly clarifying the array of aberrant molecular pathways in chordoma cells. Brachyury, as a relatively unique hallmark and more stable signaling factor in chordomas, is discovered in 2006 and is classified as a notochordal factor with associations to several pathological mechanisms of the disease. This transcriptionfactor, holds much promise as a biomarker and potential target for therapy. With the discovery of notochordal marker brachyury, the authors argue that there might be potential in further investigating of other notochordal transcription-factors. The paucity of studies performed on the level of epigenetics and other unexplored research fields is also touched on.

In chapter 3, the authors support the notion of the notochord being a crucial tissue for chordoma research. In detail, we describe a process by which we isolate notochordal tissue from the human embryonic intervertebral discs. Tissue collected from aborted fetuses with the gestational age of 9, 11, and 13 weeks were collected. After dissection, the spinal column was fixed in 3.7% formalin for 48 hours before being embedded in paraffin by standard procedure. Immunohistochemically stained tissue of the fetal vertebral spine for brachyury expression, resulted in strong staining of the notochord, but also weak staining of the intervertebral disc and vertebral body. Using laser capture microdissection, the notochordal section is isolated and good quality of RNA distracted from this section is confirmed. The
authors discuss isolation of other gestational ages and suggest this protocol to be the new standard in isolation of notochordal tissue.

Using the collected intervertebral discs discussed in the previous chapter, in chapter 4, we report on technical specifications and requirements for isolating and comparing protein samples in proteomic analysis. Total protein extraction, as well as specific TiO2-based phosphopeptides enrichment and hydrazide-based glycopeptides purification are described in detail. For quantitative differential protein expression, isobaric labeling with isobaric tag for relative and absolute quantitation (iTRAQ) is combined with liquid chromatography–mass spectrometry (LC-MS). The vast amount of data gathered from these analysis, represent a challenge for the investigator. The authors suggest the usage of several bioinformatics tools, depending on the preferred application. Software using gene enrichment with tabular results (DAVID, gProfiler, etc.) and gene enrichment with graphical presentation (String, Cytoscape) might prove to be helpful. However, as these results depict the association of genes, with protein-protein libraries not readily available, validation of such results remains a crucial aspect.

Chapter 5 sets out to use such a proteomic assessment to answer a pivotal question in chordoma, and to a lesser extent in chondrosarcomas; “Can we find biomarkers relating to tumor recurrence and do we have potential therapeutics to target them?”. Here, we have pooled tumor lysates from patients in specific groups: primary chordomas, primary chordomas that recurred, primary chondrosarcomas, and primary chondrosarcomas that recurred. With the use of TiO2 phosphopeptide enrichment protocols, similar to ones described in chapter 4, the four groups of iTRAQ labeled phosphopeptides were compared after tandem mass spectrometry analysis. Differential expression of proteins between the tumors that have recurred plotted against tumors that haven’t, demonstrates a set of biomarkers that are potentially associated with recurrence. In chordoma that recurred, increased phosphorylation of the discovered peptide could be tracked down to an increase of kinase activity in the tyrosine-protein kinases feline sarcoma oncogene (FES) and feline encephalitis virus-related kinase (FER). With regards to drug sensitivity, the set of phosphorylated chordoma proteins of recurrent patients were predicted to be targeted by Nilotinib and Imatinib. Future studies have to validate this proof of concept.

To summarize chapter 6, the authors examined cell cycle biomarkers in chordoma patients treated in our institution. Twenty-five formalin fixed paraffin-embedded chordoma specimens are immunohistochemically stained with antibodies for the cell-cycle markers protein 53 (p53), cyclin dependent kinase 4 (CDK4) and murine double minute 2 (MDM2). Extent of the staining was semi-quantitatively scored on number of positive cells (nuclear expression) categorized as 0 (Negative), 1 (1–10%), 2 (10–50%) and 3 (>50%) and quantitatively scored using optical density measurements. Clinical variables, (e.g. age, gender and overall survival) and pathological outcomes scores (e.g. necrosis, mitotic index and Ki67 scores (MIB1-LI)) were also recorded. All three cell cycle markers showed a significant correlation with MIB1-LI, although the MIB1-LI along with patient age, gender, tumor location
failed to show a relation with survival. Expression of CDK4 ($p=0.02$) and P53 ($p<0.01$), however, were both significantly correlated to poor overall survival. Also, histologically observed necrosis ($p<0.05$) and a dedifferentiated tumor subtype ($p<0.01$) were related to adverse patient outcome. These results advocate a direct or indirect link of these biomarkers with worse outcome in patients with chordoma tumors.

The final research chapter, chapter 7, concerns a novel hypothesis of viral involvement in the etiology of chordomas. As previously mentioned, a recent assumption in the etiology of chordomas, is chromothripsis. Viral involvement is frequently heralded as an event preceding this phenomenon. To examine the theory of viral involvement in chordoma, the authors report on a study in which the presence of multiple oncogenic viruses were assessed in chordoma and chondrosarcoma patients. As the most outstanding finding, the presence of parvovirus B19 (PVB19) DNA was detected in 4 of 18 chordomas (22%) and in 1 of 15 chondrosarcomas (7%). Immunohistochemical analysis recognized the VP2 capsid protein of PVB19 in 44% of cases (14 of 32), suggesting an even higher percentage of tumors affected by the virus. DNA from other viruses such as human herpes virus 7 (HHV-7) and Epstein-Barr virus (EBV/HHV4) were present in 6 of the 18 (33%) and 4 of the 18 (22%) chordoma samples. Although a thorough genome-wide search of viral DNA would increase understanding of this hypothesis, this study potentiates a viral involvement in the etiology or pathophysiology of chordoma.

In the final chapter, the authors address the important findings and place them into a general scope of chordoma research. Firstly, a general description of the clinical challenges is briefly touched on to highlight the need for biomarker research. We challenge certain important etiologic and pathophysiological concepts, such as the notochordal origin and the significance of brachyury expression and chromothripsis. Due to lack of typical notochordal biomarkers, we finally accept a notochordal origin for these tumors as “most applicable” until a notochordal expression profile is recognized. Also, we advocate the established use of methylation profiling in unraveling the (epi)genetic fingerprint that points to the hallmark of chordomagenesis. We further highlight the amount of biomarker research performed and the paucity of these results being validated on larger data sets. This by itself addresses the great challenge most chordoma researchers stumble on; the unfortunate small number of patients and cases when compares to other more frequent occurring tumors, such as breast and colon cancer. Most of the biomarker study-result, including ours, therefore must be placed into a different perspective. However, it is still required of researchers, especially in a small research area like chordomas, to use a holistic understanding of the disease, including the paucity of high quality data, and to apply this knowledge for future studies. Amalgamating unexplored zones of tumor biology and multiple fields of cell biology, would help discover molecular (prognostic) biomarkers that might advance as molecular targets for medical therapy. This way, we hope to lift the burden that physicians and patient face in the clinical management of these tumors.