

Review

A brief review of chordomas: pathogenesis, prognostic factors and therapeutic targets

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Summary. Chordomas are rare but locally aggressive cancer, which originate from primitive notochord remnants. Guidelines have recently been proposed to include the option of choosing chordomas in different locations. However, there is still a great challenge in the modern management of chordomas, primarily due to the high recurrence rate and poor prognosis. On this basis, there is a high demand for new therapeutic approaches and reliable prognostic factors. Recent progress in studying the molecular basis of this specific type of cancer has deepened the understanding of this mechanism, which overall facilitates the discovery of specific biomarkers or indicators of the disease. It also gives rise to potential targeted therapies against chordomas as evidenced by the fact that some RTK inhibitors in a clinical context have been evaluated in relation to chordomas. This article summarizes these achievements including the studies relative to pathogenesis, prognostic factors, and targeted therapies for chordomas. The theme of existing problems is also mentioned, which would facilitate general future efforts in this field.

Key words: Chordoma, Pathogenesis, Prognostic factors, Therapeutic targets, Brachyury

Introduction

In the 1890s, the concept of chordomas was originally introduced by Ribbert on the basis of the notochord hypothesis (H., 1894). Chordomas are able to develop anywhere in the spine. There are different hypotheses regarding distribution. Some reported an almost equal distribution in the clivus, sacrum/coccygeum, and along the mobile spine (McMaster et al., 2001; Ahmed et al., 2010), while others reported these tumors to be observed more frequently in the sacrum/coccygeum compared to the clivus (Heffelfinger et al., 1973; Chugh et al., 2007). In the past, it was difficult to recognize chordomas since histological patterns could be rather confusing to differentiate. Their features on magnetic resonance imaging (MRI) and computed tomography (CT) scanning are particular, but they are not specific. Moreover, they can be easily confused with chondrosarcomas in radiologic diagnosis (Almefty et al., 2007). Fortunately, given the efforts of pathogenetic studies, the immunohistological method allows a clear differentiation through the help of the most specific and sensitive markers, such as brachyury (Vujovic et al., 2006; George et al., 2015).

As a rare form of cancer, chordomas only account for less than 4% of all primary malignant bone tumors (McMaster et al., 2001) and has an overall median survival rate of approximately 6 years (Chugh et al., 2007). Nonetheless, chordomas are locally aggressive in spite of the infrequent incidence and the slow-growing nature present in this form of cancer. Moreover, they are

generally characterized by their high recurrence rate and poor indication of outcomes, which together, constitute a great challenge in the modern management of this disease. Nowadays, chordomas have drawn increased levels of attention from researchers all over the world. This could be partially evidenced through bibliometric analysis based on PubMed (Fig. 1), which showed that compared to other disease-related publications, there is a relatively higher increase chordoma focused studies (e.g. 2014, 2017, 2018).

Although consensus was reached in regard to chordoma research approaches (Stacchiotti et al., 2015), there are still many questions to address relative to the disease, especially regarding the prognosis and treatment (George et al., 2015). On the one hand, although a variety of different molecular factors have reportedly correlated with the clinical outcomes, there is no reliable prognostic biomarker identified to date. On the other hand, recurrence often occurs among chordoma patients receiving the standard clinical care of en bloc resection in conjunction with radiation therapy (Patel and Schwab, 2016). Worst of all, recurrent chordomas are nearly impossible to eradicate.

Even through the utilization of conventional treatments, problems are still present. The subtotal resection has a risk of giving rise to further recurrence (Kayani et al., 2014), while wide-margin resection leads to a loss of function in the nerves (Patel and Schwab, 2016). In addition, it is often challenging to make the wide-margin resections due to the rather complicated locations in which chordomas are typically found in comparison to other different tumor types. Radiation therapy, such as photon/proton beam radiation and carbon ion therapy (DeLaney et al., 2009, 2014; Imai et al., 2016; Indelicato et al., 2016), for example, introduces the potential risk of adverse effects despite therapeutic benefit that is possible. Moreover, there is an inherent risk of sacral insufficiency fractures (Patel and Schwab, 2016). Additionally, there is no consensus as to whether it is best for chordomas to be irradiated preoperatively or postoperatively. However, DeLaney et al. reported that stand-alone irradiation of various chordomas with proton-beams led to disease stability in

approximately 80% of cases after a median of 4 years of follow-up treatment (DeLaney et al., 2009).

Currently, there are different guidelines determined to locate and remove chordomas depending on the area of the body. For example, intracranial resection is preferred to remove chordomas in the base of the skull or cervical spine to give the maximum clearance and ensure that the nerve function and quality of life of the person is not compromised. Moreover, wide en-bloc resection has been adopted to remove chordomas in the thoracolumbar and sacro-coccygeal regions below S4. However, alternatives such as a stand-alone (proton or heavy ion) irradiation treatment need to be discussed with the patient in cases of sacro-coccygeal chordoma removal above S4 and in other situations, such as an expected loss of function of important nerve-roots (Stacchiotti et al., 2015).

For patients with recurrent chordomas, treatment alternatives are often limited to “salvage” treatment options with curative intent for patients with isolated lesions. These types of treatments include salvage resection, salvage radiotherapy, and salvage palliative/supportive, while the best palliative care is used for those with multifocal recurrences (Stacchiotti et al., 2017). Contrastingly, the progress being made with systemic therapy is very limited. For example, there is no therapeutic agent with proven efficacy (Diaz and Cusimano, 2011). Minimal advancements have been made for chordomas compared to the treatments that exist for other types of cancer, such as breast and lung cancers. Additionally, small-molecule drugs have not yet been established for any kind of chordoma (Patel and Schwab, 2016). On these bases, there is a high demand for new therapeutic approaches (Scheil-Bertram et al., 2014).

Recent progress in understanding the molecular basis of chordoma mechanisms has shed lights on solving these difficulties. Deepening the insight into pathogenesis seems promising in order to better understand a number of specific biomarkers as well as prognostic factors. It also gives rise to prospective advancements being made towards targeted treatments against chordomas by revealing the most relevant

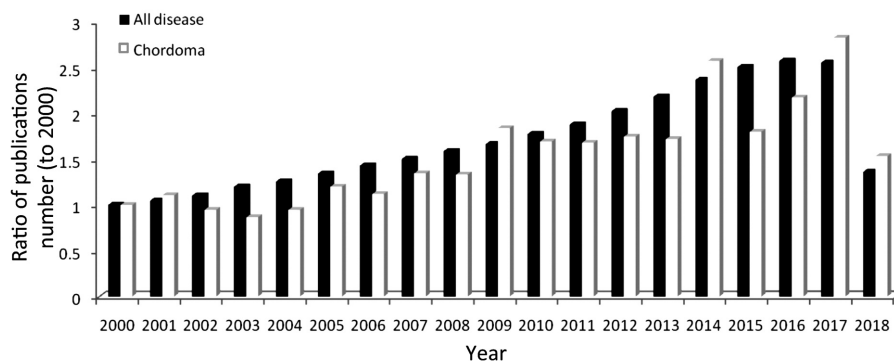


Fig. 1. Graph displaying bibliometric analysis based on PubMed from 2000 to 2018 comparing the keyword setting of “chordoma” and “all disease”.

signaling pathways. A variety of anticancer drugs in clinical use have been evaluated in the context of chordomas, and these drugs are known to target the important signaling pathways, which are closely linked to the development of the disease (Di Maio et al., 2015). In recent studies, we briefly summarized these advancements being made as well as discussed existing problems, which would help to facilitate the future efforts in this field.

Pathogenesis

Broadening the understanding of chordoma pathogenesis will ultimately lead to the identification of novel prognostic markers as well as targeted therapeutic strategies. Although there is still a lot of room for advancements to be made, significant achievements in previous studies of mechanism have been introduced (Sun et al., 2015; Youssef et al., 2016). All in all, we have gained an overview of the results with increased interest in the new discoveries being made.

Disease origin

As early as 1858, the first hypothesis about the origin of chordomas was gathered based on the histological appearance, which states that chordomas are of notochordal origin (Müller, 1858). Later, it was proposed that hamartomatous lesions of notochordal origin could serve as a precursor of chordoma development and can be considered as benign counterparts (Ribbert, 1894). Up to this point, it has been widely accepted that chordoma cells come from the remnants of the embryonic notochord (Nibu et al., 2013), which is a transient structure that display critical developmental roles in humans (Corallo et al., 2015). After inducing vertebral column formation, the notochord disappears (Kreshak et al., 2014). Given the greater occurrence of notochordal remnants compared to the incidence of chordomas in humans, it was suggested that the notochordal remnants mainly stay dormant but transform into malignancies under stimulation or pressure, such as environmental factors, gene mutations, or other potential triggers (Choi et al., 2008).

Furthermore, Yamaguchi et al. investigated the link between persistent notochordal remnants and chordomas. First, the site of the vestiges closely corresponds with how the chordomas are distributed. Second, there are considerable morphological similarities under the observations of both transmitted electron microscopy and light microscopy. Third, significantly overlapped immunophenotype between notochordal remnants and chordomas exist (Yamaguchi et al., 2004). Moreover, Vujovic et al. also suggested the origin of chordomas from notochordal remnants by detecting the molecule of brachyury in both chondroid and chordoid components of chordomas, which represents the specific link between chordomas and the notochord for the first time (Vujovic et al., 2006; Nibu et

al., 2013). Nucleus pulposus develops from the embryologic notochord; *in vivo* studies showed that the embryonic notochord directly gives rise to all types of cells present in the nucleus pulposus (Choi et al., 2008). Injury to the nucleus pulposus of intervertebral disks may cause a predisposition to developing chordomas (Mayer and Donnelly, 2013). Alholle et al. performed DNA methylation that profiled a total of 26 chordomas and normal nucleus pulposus samples and found numerous significantly different cancer-specific hypermethylated genes (Alholle et al., 2015).

Transformation drivers

Unanswered questions still remain in regards to the transformation of notochordal cells to the benign or malignant variants. The theory of cancer stem cell research provides possible explanations (Sun et al., 2015), which indicate that a small subpopulation of tumor cells exhibiting cancer stem cell characteristics could serve as the driving force behind tumor growth and differentiation (Aydemir et al., 2012). As evidenced by Hsu et al., the formation of sarcospheres in the human chordoma cell line has been identified. It was also found that chordoma sarcospheres were able to self-perpetuate and exhibit higher expressions of the functional stem cell marker, which is different from classic chordoma cells (Hsu et al., 2011a). Moreover, sarcospheres were found to differentiate into other types of cells, which implies a hierarchical model for chordoma cell transformation and differentiation (Sun et al., 2015). Leukemia inhibitory factor, which has multiple functions in stem cell biology, was found to induce cancer stem cell phenotypes in chordomas. Similarly, aldehyde dehydrogenase 1 activity has been used as a functional stem cell marker, which have been found in chordomas (Lohberger et al., 2012; Gulluoglu et al., 2017). Moreover, it has been discovered that chordoma cells have a cancer stem cell subpopulation that can be identified by the stem cell markers CD24, CD133, CD15, and ALDH (Fujii et al., 2016). However, given the fact that no *in vivo* studies were conducted to test these hypotheses, there is a lack of validation for the stem cell hypothesis, which calls for further research to be done in the future.

Molecular basis

Despite the previously mentioned knowledge that has been unveiled, the molecular events in the beginning and developmental stages of chordomas have not been fully illustrated, especially regarding the differentially expressed genes involved in chordoma origin. Numerous studies have been conducted to explore the proteins implicated in the preservation of the embryonic structure in chordomas. Among them, the most significant and novel discovery has been the transcription factor brachyury, which accounts for the initiation and progression of chordoma cells (Vujovic et al., 2006; Sun et al., 2015).

Brachyury is encoded by the human T gene, and represents one of the key regulators of notochord formation during embryogenesis (Kispert et al., 1995). Brachyury fulfills the essential role in notochord formation via the synergistic interaction between other transcription factors, such as members of the Fox family (Sun et al., 2015). The significant effects of gene overexpression and knockdown for brachyury on cell proliferation support the important role in chordoma growth and progression (Fernando et al., 2010; Hsu et al., 2011b; Presneau et al., 2011; Nelson et al., 2012). Moreover, Yang et al. found that T (*brachyury*) gene duplication confers major susceptibility to familial chordomas (Yang et al., 2009b). Pillay et al. also reported that the discovery of a common single-nucleotide variant in T genes are strongly associated with chordomas (Pillay et al., 2012).

Besides brachyury, the upstream signaling pathways also play essential roles. For instance, during the early embryonic development, the notochord produces secreted factors to guide the organogenesis, such as the sonic hedgehog pathway, Wnt/ β -catenin, and fibroblast growth factor (FGF) (Nibu et al., 2013). The aberrant activation of these signaling pathways could contribute to chordomagenesis.

Among them, the sonic hedgehog is a crucial morphogen secreted by the fetal notochord that directs embryonic patterning of the neural tube and adjacent sclerotomes (Chari and McDonnell, 2007). Reactivation of the sonic hedgehog pathway in notochordal cells or benign notochordal tumor cells may represent a potential mechanism for notochordal cell survival and neoplastic transformation (Cates et al., 2010).

It has been disclosed that brachyury is a direct target of Wnt *in vivo* (Yamaguchi et al., 1999). Consequently, the possibility that the Wnt/ β -catenin signaling pathway could play a role in regulating brachyury has attracted researcher's attention (Arnold et al., 2000). The expression of the Wnt/ β -catenin signaling pathway was detected in numerous chordomas (Naka et al., 2001; Horiguchi et al., 2004; Triana et al., 2005; Aviel-Ronen et al., 2016). Moreover, the pathway activation has also been observed in chordomas (Diaz et al., 2012; Long et al., 2013; Alholle et al., 2015; Chen et al., 2017b). However, the role of this pathway as well as the effects of pathway inhibition on chordoma patients is not yet fully analyzed.

FGF is closely involved in the pathophysiology of chordomas. Hu et al. found neutralization of FGF2 and was able to reduce phosphorylated MEK/ERK and the expression of brachyury (Hu et al., 2014). It also induced significant apoptosis and potently inhibited overall cell growth. Combined with selective inhibition of phosphorylated FGFR, MEK, and ERK in addition to the knockdown of brachyury, research concluded that the FGFR/MEK/ERK/brachyury pathway regulates the cell growth and survival of chordomas (Hu et al., 2014). Besides, it was also suggested that the signaling cascade

may represent a novel therapeutic target for chordomas.

In addition to brachyury and related signals, a number of molecular analyses also revealed other genetic abnormalities in chordoma pathogenesis. It has been recognized that the aberrations in the cell cycle lead to tumorigenesis and chordoma progression (Naka et al., 2005; Kaloostian and Gokaslan, 2014). For example, Yakkoui et al. observed the significant correlation between the expression of CDK4, p53 and poor overall recovery in chordoma patients (Yakkoui et al., 2014).

The receptor tyrosine kinases (RTKs) and their related secondary messengers also play key roles in chordoma development (Tamborini et al., 2006; Heymann and Redini, 2013). Previous research demonstrates that numerous RTKs exhibiting dysregulation in the expression and phosphorylation plays an important role in chordoma growth. For example, platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), HER2 (Neu), and c-Met, as well as their downstream signals including phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT), and mammalian target of rapamycin (mTOR) (de Castro et al., 2013) are linked to chordomas. There has been evidence for the activation of PI3K/RAS/AKT/mTOR signaling in chordoma cells (Presneau et al., 2009). Consistently, key downstream proteins within the pathway were shown to be phosphorylated in human chordomas (Schwab et al., 2009a). A study that was conducted with kinase antibody array revealed that the consistent activation of AKT experienced the highest phosphorylation in chordomas (Dewaele et al., 2011). The recurring activation of EGFR and mTOR as well as the frequent loss of PTEN gene loci implies the important mediation of the PI3K/AKT pathway in chordoma transformation.

Though the rare occurrence of chordomas pose a great challenge to the pathogenesis study because these achievements in understanding the molecular alterations related to chordomas greatly facilitated the discovery of prognostic markers and potential targets. Ultimately, those achievements have paved the way to the development of targeted therapies and will be discussed in the following sections.

Prognostic factors

Nowadays, the lack of reliable prognostic markers represents the most significant challenge to overcome in chordoma management, and various efforts have been made for this end goal. To date, most reports on chordoma prognosis were retrospective cohort studies with immunohistochemical staining of tumor tissue samples. As a result, a large collection of biomarkers was identified. Among them, the tissue protein markers were the most widely studied factor, followed by genetic, immunological, and inflammatory markers which were delineated by the previous review (Zou et al., 2016b).

The role of brachyury

Being the most significant discovery within the context of molecular basis for chordomas, brachyury has been identified as a unique, specific diagnostic marker of chordoma development (Vujovic et al., 2006; Barresi et al., 2014). The extensively observed immunopositive signals for brachyury protein expression indicates that there is a potential central role in the growth regulation of the majority of chordomas found in the base of the skull (Kitamura et al., 2013). Furthermore, Vujovic et al. found that brachyury was restricted to chordoma cells at a high protein expression in all 53 primary chordoma tissue samples, but not in a wide variety of other neoplasms or normal tissues (Vujovic et al., 2006). The unusually high expression of brachyury in chordomas also shows the differentiation from other tumors with similar histology and geographical location, thus providing an ideal diagnostic adjunct (Romeo and Hogendoorn, 2006; Schwab et al., 2009b; Jo et al., 2014). Additionally, brachyury seems promising in targeted drug development given the fact that it is normally silenced in post-developmental tissue but aberrantly re-expressed at high levels in chordomas (Vujovic et al., 2006). Such potential could be partially supported by the interesting finding that the brachyury gene silencing in chordoma cells resulted in the complete cell growth arrest and senescence (Hsu et al., 2011b). This will be further discussed in the targeted therapies section.

Unfortunately, despite the above evidence, brachyury expression failed to be established as a prognostic indication in chordomas. In 2013, Zhang et al. analyzed tumor tissues on a large scale with high-throughput tissue microarray technology, and found no significant relationship between the brachyury expression and clinicopathologic parameters, such as overall survival rate in a population of 78 chordoma patients (Zhang et al., 2013). Given the relatively low frequency in observing the brachyury expression, the differences in the sensitivities and experiment criteria should be fully considered. Nonetheless, additional studies with a larger sample size are highly encouraged.

The role of RTK signaling pathways

As stated above, RTKs and downstream effectors play essential roles in chordoma pathogenesis. It has been shown that chordomas express activated platelet-derived growth factor receptor (PDGFRB) as well as EGFR and c-MET (Weinberger et al., 2005). For instance, Akhavan et al. found that the expressions of c-MET, PDGFR- α and EGFR correlated with the prognosis in different trends among 104 recurrent lesions within spinal chordoma patients (Akhavan-Sigari et al., 2014a). Furthermore, the targeted treatment with small-molecule inhibitor benefits a number of patients (Tamborini et al., 2006). The same group also disclosed that all 80 recurrent chordoma lesions displayed strong

expressions of VEGFR-2 (Akhavan-Sigari et al., 2014b). Zhang et al. reported that Raf-1 expression, rather than ERK1/2, was related to shorter continuous disease-free survival time (Zhang et al., 2015). De Castro et al. demonstrated that the high expression of phosphor-AKT is associated with poor prognosis (de Castro et al., 2013). In agreement, Chen et al. revealed that AKT2 had high expression levels in most chordoma patient samples, which renders patients vulnerable for relapse (Chen et al., 2015). Other groups detected significant differences in the expression of mTOR and PTEN between sacral chordoma and normal tissues, which correlates with tumor invasion (Chen et al., 2014). The presence of activated receptor tyrosine kinases (RTKs) leads to the activation of secondary transducers belonging to the MAPK or PI3K/AKT pathways, which activate mTOR; downstream mTOR, S6K/S6, and 4E-BP1 harmonize protein synthesis, and promote cell growth and proliferation.

Other permission factors and existing problems

Other promising factors under investigation for predicting the recurrence include the following: Stat3 (Yang et al., 2009a), PHLF (Chen et al., 2015), hTERT (Zou et al., 2016a), surviving protein (Chen et al., 2013), SPHK1 (Zhang et al., 2014), IMP3 (Zhou et al., 2014), PARP1 (Zou et al., 2016c), CSPG4 (Schoenfeld et al., 2016), CD40 (Li et al., 2013), as highlighted by Zou et al. (Zou et al., 2016b). Most recently, Ma et al. reported overexpression of the oncogene inhibitor of the apoptosis-stimulating protein p53 (iASPP) in chordoma patient samples, which was associated with tumor invasion, local recurrence, and was indicative of poor prognosis. They also observed the reduced proliferation and invasion of increased cisplatin sensitivity in iASPP-silenced cells (Ma et al., 2017). Chen et al. combined chordoma tissue microarray and IHC analysis to illustrate the upregulated expression of sex-determining region Y (SRY)-box 9 (SOX9) and how it correlated to poor prognosis (Chen et al., 2017a). SOX9 inhibition was shown to have potent anti-proliferative abilities and synergic effects with therapeutic drugs. Additionally, aberrant miRNA expression has been recognized to affect chordoma pathways (Long et al., 2013; Duan et al., 2014; Sun et al., 2015). Duan et al. indicated the prognostic significance of miR-1 expression in chordoma patients (Duan et al., 2014). Gulluoglu et al. uncovered the potential role of miR-31, miR-140-3p, miR-148a, and miR-222-3p in chordoma initiation and progression by regulating numerous protein targets, like RDX, MET, DNMT1, DNMT3B, TRPS1, BIRC5, and KIT (Gulluoglu et al., 2016) for example. Similarly, Bayrak et al. also observed that the same genes that had been differentially expressed in chordomas compared with a healthy nucleus pulposus having miRNA profiling, which facilitated the delineation of the differential regulation of cancer-related genes in chordomas (Bayrak et al., 2013). Additionally, Chen et

al. revealed human miR-185-5p as a crucial miRNA in chordoma development via the Wnt signaling pathway (Chen et al., 2017b). These new findings expanded the current knowledge relative to potential targets and biomarkers used to determine chordomas. These results are all summarized within Table 1.

Notably, Zhou et al. observed the significance of ENO1, PKM2, and gp96 expressions in chordoma prognosis with univariate analysis, which was not seen by multivariate analysis (Zhou et al., 2010). This suggests that specific recurring protein overexpression may not be regarded as the indication of an independent and reliable prognostic factor in chordomas (Zhou et al., 2010), which is a similar result to that observed in comparison to different studies. For instance, Chen et al. found that positive expressions of VEGF and MMP-9 could indicate the local recurrence of sacral chordoma (Chen et al., 2011). Naka et al. suggests that a high expression of MMP-2 rather than MMP-9 was an indicator of an unfavorable clinical outcome as illustrated through the result of 29 patients with non-skull based chordomas (Naka et al., 2004). Contrastingly, Li et al. indicated that the prognosis of chordoma patients was not significantly linked to VEGF and MMP-2 gene expression (Li et al., 2012). This could be partially attributed to the relatively small number of patients involved in the investigations, which was believed to be prone to yielding overestimated results or effects (Thorlund et al., 2011). Although a variety of factors have been proposed in the context of chordoma prognosis, the results are still either inconclusive or controversial (Zou et al., 2016b). To date, there is no

reliable or established prognostic biomarker of chordomas.

Targeted therapies

Targeting brachyury and related pathways

Based on the progress that has been made in understanding the molecular basis of chordomas in oncogenesis, targeted chemotherapy is now an emerging treatment (Han et al., 2009; Presneau et al., 2009). Targeting brachyury has been regarded as an attractive method of treating chordomas, primarily because it plays an important role in driving cancer behavior and is not expressed in normal, non-chordoma tissue (Di Maio et al., 2015). Furthermore, a brachyury inhibitor has already been tested *in vitro* and displayed favorable results overall (George et al., 2015). Encouragingly, enough, in a recently completed phase I study, the drug that was directed against brachyury has been administered safely in patients with chordomas and it was shown to be able to activate brachyury-specific T cells, which rationalized the unique strategy to target the mesenchymalization process for cancer treatment (Heery et al., 2017).

In addition to strategies that focused on directly targeting brachyury, deactivating its downstream or interacting signaling pathways is also promising to prevent proper function, which therefore provides an alternative approach (Di Maio et al., 2015). For instance, FGFR/MEK/ERK cascade was reported to mediate the downstream signaling of brachyury in chordomas and

Table 1. List of relevant research that highlights prognostic factors and/or therapeutic targets as mentioned in this study.

Name	Reference	Annotation
brachyury	Vujovic et al., 2006; Kitamura et al., 2013; Barresi et al., 2014	Prognostic factor studies
	George et al., 2015; Heery et al., 2017; Magnaghi et al., 2017	Therapeutic target studies with drugs in treatment, e.g. afatinib
c-MET	Akhavan-Sigari et al., 2014a	Prognostic factor studies
PDGFR	Akhavan-Sigari et al., 2014a	Prognostic factor studies
	Casali et al., 2004; Stacchiotti et al., 2012	Therapeutic target studies with drugs in treatment, e.g. imatinib
EGFR	Akhavan-Sigari et al., 2014a	Prognostic factor studies
	Linden et al., 2009; Singhal et al., 2009; Launay et al., 2011; Siu et al., 2013; Scheipl et al., 2016; Magnaghi et al., 2017	Therapeutic target studies with drugs in treatment, e.g. cetuximab, gefitinib, erlotinib, lapatinib, afatinib
VEGFR-2	Akhavan-Sigari et al., 2014b	Prognostic factor studies
	Asklund et al., 2014	Therapeutic target studies with drugs in treatment, e.g. erlotinib, bevacizumab
Raf-1	Zhang et al., 2015	Prognostic factor studies
AKT	de Castro et al., 2013; Chen et al., 2015	Prognostic factor studies
mTOR	Chen et al., 2014	Prognostic factor studies
PI3K/AKT/mTOR	Han et al., 2009; Schwab et al., 2009a; Stacchiotti et al., 2009; Davies et al., 2014	Therapeutic target studies with drugs in treatment, e.g. PI-103, rapamycin, sirolimus, and MLN0128
PTEN	Chen et al., 2014	Prognostic factor studies
iASPP	Ma et al., 2017	Prognostic factor studies
SOX9	Chen et al., 2017a	Prognostic factor studies
FGFR/MEK/ERK	Hu et al., 2014	Therapeutic target studies with drugs in treatment
CTLA4	Yang et al., 2010	Therapeutic target studies with drugs in treatment, e.g. ipilimumab
miRNAs	Duan et al., 2014	Prognostic factor studies

have positive feedback related to treatment methods (Hu et al., 2014). These render FGFR/MEK/ERK signaling pathway components, which are potential targets. Nelson et al. reported the interaction that exists between brachyury and the EGFR signaling pathway (Nelson et al., 2012).

Targeting RTK signaling pathways

Receptor tyrosine kinases (RTKs), and their downstream effectors, are additional potential targets. Currently, there are several inhibitors that can be used to treat chordomas, which include PDGFR (imatinib), EGFR (cetuximab, gefitinib, erlotinib), and mTOR (sirolimus) inhibitors (Casali et al., 2007; Barry et al., 2011). Tamborini et al. detected the PDGFR- β expression and phosphorylation in chordomas (Tamborini et al., 2006). Imatinib, which displays inhibitory potency against PDGFR, has shown modest activity in patients who experience advanced chordomas (Casali et al., 2004). This prompted a phase II study of 56 advanced chordoma patients to realize the overall clinical benefit in 64% of patients and to stabilize the disease in 70% of patients (Stacchiotti et al., 2012). Furthermore, the co-expression and co-immunoprecipitation of PDGFR- β and EGFR have been described at the preclinical level. (Tamborini et al., 2010; Dewaele et al., 2011) and the immunoreactivity of EGFR has been reported in chordoma (de Castro et al., 2013). Phosphor-EGFR expression was observed in a large proportion of chordomas and is associated with aggressive clinical behavior (Weinberger et al., 2005; Fasig et al., 2008; Ptaszynski et al., 2009; Shalaby et al., 2011). *In vitro* studies showed that EGFR inhibitors, such as erlotinib and gefitinib, have proliferative inhibition against chordoma cell line (Siu et al., 2013). Antibody treatment (cetuximab) and treatment that use specific EGFR inhibitors (gefitinib or erlotinib) (Linden et al., 2009; Singhal et al., 2009) or broader-spectrum tyrosine-kinase inhibitors (lapatinib) (Launay et al., 2011; Stacchiotti et al., 2013) were shown responses and clinical improvement in recurrent sacral and skull base chordomas, implying that targeting EGFR and downstream pathways could serve as a viable option to treat chordomas. Researchers who agree with this approach, Scheipl et al., undertook a focused compound screen and identified specific EGFR inhibitors (e.g. sapitinib, gefitinib, erlotinib) as potential tools that can be used against chordoma (Scheipl et al., 2016). Most recently, notable findings have been reported by Magnaghi et al. who performed extensive kinase-inhibitor profiling against a panel of chordoma cell lines (Magnaghi et al., 2017). As a result, afatinib was the only EGFR inhibitor with activity across the chordoma panel, which could be attributed to the unique ability of afatinib to target both EGFR inhibitors and the brachyury protein. Moreover, this data rationalized the upcoming European phase II study on afatinib completed in regards to advanced chordomas (Magnaghi

et al., 2017).

However, targeting RTKs in chordomas still remains a challenge due to the multiplicity and functional redundancy. For instance, c-Met is a known bypass signaling pathway to EGFR inhibitors and tumor expressing high levels of phosphorylated Met tends to be resistant to EGFR inhibition (Scheipl et al., 2016). Fortunately, this could be addressed by simultaneous inhibition of multiple RTKs being activated or by targeting common signaling pathways downstream. For instance, EGFR and PDGFR signaling pathways converge on the PI3K/AKT/mTOR pathway, which is negatively regulated by PTEN. It was found that PI3K/AKT/mTOR components on the pathway are activated (Presneau et al., 2009; Schwab et al., 2009a; Tamborini et al., 2010) while PTEN is suppressed in tumor tissue samples from chordomas (Han et al., 2009). Preclinical experimental studies using PI3K/AKT/mTOR (PI-103) and mTOR (MLN0128) inhibitors support this strategy (Han et al., 2009; Schwab et al., 2009a; Davies et al., 2014). Stacchiotti et al. found that the mTOR pathway, in conjunction with PDGFR- β , can be activated in chordomas, and the combination of imatinib plus sirolimus may be effective in advanced imatinib-resistant chordomas (Stacchiotti et al., 2009). There is even evidence supporting the claim that over 50% of chordomas are responsive to mTOR inhibitors (Tamborini et al., 2010).

Other treatments against chordomas

Efforts are being made to profile chordomas based on their molecular structure, and applying precision oncology to individual patients has been proposed (Shrager and Tenenbaum, 2014). For example, VEGFR-2 mediated angiogenesis signaling pathways were located in some chordomas (Akhavan-Sigari et al., 2014c). On this basis, the combination of erlotinib and bevacizumab was proven to be efficient in stabilizing the disease in following clinical studies (Asklund et al., 2014). Conclusively, targeted therapies will be engaged in an increasing manner to aid in the future treatment of chordomas, either as a preoperative approach or in addition to surgery or radiation therapy.

It is also noted that great interest in chordoma immunotherapy has surfaced in recent years (Patel and Schwab, 2016). Programmed death 1 (PD-1) and its ligand (PD-L1) have been implied in promoting tumor progression (Akbay et al., 2013; Bigelow et al., 2013). PD-1 is expressed on the surface of lymphocytes and PD-L1 is expressed by tumor cells, tumor-associated immune cells, and stromal cells (Bloch et al., 2013; Fang et al., 2013). PD-L1 expression is a dynamic process that correlates with changes made to the tumor microenvironment. PD-L1 is responsive to the presence of Th1 related cytokines (IFN- γ), and it can be induced through the activation of the TLR4 or STAT pathways (Loke and Allison, 2003). Mathios et al. reported the presence of PD-1 and PD-L1 pathways in chordomas for

the first time and showed that primary chordoma tissue indicates a variable expression of PD-1 and PD-L1 in the infiltration of immune cells (Mathios et al., 2015). It was also suggested that post-transcriptional regulation of PD-L1 mRNA transcripts may play a fundamental role in modulating activity in the PD-1 pathway (Mathios et al., 2015). Zou et al. assessed the expression levels of PD-1 and PD-L1 in a patient group containing 54 spinal chordoma patients. This research denoted that tumors with positive PD-L1 expression levels were linked to having advanced stages of chordoma development and tumor-infiltrating lymphocytes (Zou et al., 2016c). Cytotoxic T lymphocyte antigen 4 (CTLA4) is a negative regulator of the immune response and has been identified as a target to prevent chordomas (Walunas et al., 1994). The CTLA4-specific monoclonal antibody, ipilimumab, was the first immunological cancer therapy that was approved by the FDA (Pardoll, 2012).

Conclusion

Although resection and radiation therapy treatments constitute the standard practice of treating chordomas, problems still remain, such as poor prognosis and a lack of effective systemic therapies. Recent advances in understanding the molecular basis of chordoma development revealed numerous potential biomarkers and pathways found in chordomas. This exhibits close participation in or essential contribution to chordoma pathogenesis, including brachyury, receptor tyrosine kinases, and mediated signaling pathways. Significant advances have been made in distinguishing specific biomarkers (e.g. expression or phosphorylation) and correlating them with clinical outcomes on the basis of a limited number of patients, which is prone to yielding overestimated results or effects. This can be partially evidenced by the fact that there is no reliable prognostic factor for chordomas so far, despite the wide variety of prospective biological markers that have been determined. Nonetheless, the efficacy of some clinical RTK inhibitors against chordomas can be regarded as a good start for the development of finding targeted chemotherapy treatments for this rare disease. Overall, the efforts being made in exploring the molecular mechanism and novel targets for chordomas are extremely beneficial.

Competing interests. The authors declare that they have no competing interests.

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