Epigenetic deregulations in chordoma

Xin Yu and Zheng Li

Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100042, China

Received 20 April 2015; revision accepted 22 June 2015

Abstract

Chordoma is a rare type of malignant bone tumour arising from remnant notochord and prognosis of patients with it remains poor as its molecular and genetic mechanisms are not well understood. Increasing evidence has demonstrated that epigenetic mechanisms (DNA methylation, histone modification and nucleosome remodelling), play a crucial role in the pathogenesis of many diseases. Aberrant epigenetic patterns are present in patients with chordoma, indicating a potential role for epigenetic mechanisms in this malignancy. Furthermore, epigenetic alterations may provide novel biomarkers for diagnosis and prognosis as well as therapeutic targets for treatment. In this review, we discuss relevant epigenetic findings associated with chordoma, and their potential application for diagnosis, prognosis and treatment.

Introduction

Chordoma is a rare malignant neoplasm of bone arising from remnant notochordal tissues. It usually arises in the axial skeleton, 50% of these tumours being located in the sacrum (1). Surgery remains the best standard treatment, but adequate excision is difficult due to close relationships between elements of the central nervous system and other vital structures (2–4). Thus, prognosis of patients with chordoma remains poor, with local recurrence levels of over 40%; overall median survival is approximately only 6 years (5). Although morphology and immunoprofile of chordoma cells are well characterized, its molecular and genetic mechanisms are not well understood. Identification of novel molecular pathways

will contribute to finding new targets for chordoma therapy and epigenetic deregulation has recently emerged as new pathogenic mechanism.

Genetics is the study of variation in the genetic code that results in changes in phenotype (6). Epigenetics is the study of various mechanisms that result in mitotically heritable changes in gene expression that do not involve any alterations in underlying DNA sequences (7–9). Epigenetic modifications mainly include DNA methylation and histone modifications, by non-coding RNAs.

Traditionally, aetiology and pathogenesis of human diseases mainly focused on determining genetic and environmental factors. However, accumulating evidence has shown that deregulation in epigenetic networks plays significant roles in many pathological processes, particularly in cancers. The DNA sequence remains stable in different tissues but epigenetic status of the genome varies considerably. In addition, genetic alterations are not reversible, whereas the epigenetic changes are reversible with use of pharmacological agents. Several epigenetic treatment strategies have been achieved in various cancers. Thus, identifying epigenetic changes in disease may provide insights into their pathogenesis, as well as development of new therapeutic targets. This review provides an introduction into epigenetics, focusing on epigenetic changes associated with chordoma.

Overview of epigenetic mechanisms

The definition of epigenetics has undergone decades of debate and research since the term “epigenetics” was originally coined by Conrad Waddington in 1942 (10). Its most commonly used definition is ‘the study of mitotically heritable changes in gene expression without any alteration in the underlying DNA sequence’. The three main epigenetic mechanisms include DNA methylation, histone modifications and post-transcriptional gene regulation by non-coding RNA, commonly referred to as miRNA.

Correspondence: Zheng Li, Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100042, China. Tel.: +86 010 69152812; Fax: +86 010 69152812; E-mail: kleeo@163.com
Xin Yu and Zheng Li contributed equally to this work.

DNA methylation

DNA methylation is the transfer of a methyl group (-CH₃) to a cytosine nucleotide within in a given DNA sequence, which is catalysed by DNA methyltransferases (DNMTs) (11,12). DNA methylation correlates with transcriptional silencing and takes place almost exclusively at DNA sequences that contain cytosines adjacent to a guanine nucleotide (CpG site) (13,14). CpG islands are unmethylated under normal conditions except in some biological processes such as silencing of imprinted genes and X chromosome inactivation (15). There are about 30 million CpG sites in the human genome, with certain regions of DNA containing a high proportion of CpG sites, known as CpG islands (16). DNA methylation regulates various cellular processes including transcription, genomic imprinting and X chromosome inactivation. Abnormal DNA methylation is often associated with inappropriate transcriptional silencing and diseases. For example, aberrant methylated CpG islands in cancer may silence tumour suppressor genes and subsequently uncontrolled cell growth and cancer progression result (17,18).

Histone modification

Once, histones were only considered to be packaging proteins for DNA without any role in regulation of gene expression. However, since the 1990s histone modification has been identified as a branch of epigenetic regulation and the field has received much attention. The nucleosome is the basic subunit of chromatin; each is comprised of a 146-bp (base -pair) length stretch of DNA wrapped around an octamer, which consists of two copies of each of the four core histones, H2A, H2B, H3 and H4 (19,20). Histones themselves are proteins (with inherent positive charge) that bind to DNA (with its inherent negative charge). Histone modifications include addition of acetylation, methylation, phosphorylation, ubiquitination, ADP ribosylation, deimination/citrullination and proline isomerization tags (21,22). Acetylation of lysine residues is the most common type of histone modification, often resulting in transcriptional activation (23). Histone modifications are associated with regulation of many biological and pathological processes such as of the cell cycle, differentiation and apoptosis.

MicroRNAs

MicroRNAs (miRNAs or miRs) are a class of non-protein-coding RNAs of approximately 22-nucleotides, that post-transcriptionally modulate gene expression by

binding to messenger RNA, leading to transcript degradation or translational inhibition (24,25). Thus, miRNAs exert their roles by regulating target genes. It has been proven that miRNAs regulate expression of more than a third of protein-encoding mRNAs. miRNAs play significant roles in various cell processes, such as in the cell cycle, cell differentiation, apoptosis, and immune functions. Moreover, deregulated miRNA expression is involved in many diseases, specially cancers (26,27). They are frequently deregulated in malignancies and function as oncogenes (when up-regulated) or tumour suppressors (when down-regulated) (28).

Epigenetic alterations in chordoma

The earliest evidence for epigenetic influence in cancers comes from gene expression and DNA methylation studies. Major epigenetic mechanisms implicated in the pathogenesis of cancers (DNA methylation, histone modification and post-transcriptional gene regulation by non-coding RNA, miRNA) have also been involved in chordoma pathogenesis.

More importantly, as recent pre-clinical successes of inhibitors against bromodomain-containing protein 4 (BRD4, acetyl-lysine chromatin-binding protein), have entered pre-clinical trials, epigenetic cancer therapies have accrued more attention (29–31).

Aberrant DNA methylation in chordoma

Since aberrant DNA methylation was first identified as cancer-related epigenetic alteration, increasing cancer-related epigenetic changes have been observed, including hypermethylation of tumour suppressor genes and genome-wide DNA hypomethylation (18,32,33). Aberrant DNA hypermethylation takes place mainly in unmethylated CpG islands, located in promoters of many tumour suppressor genes (TSGs). The hypermethylation leads to silencing of TSG expression and loss of normal TSG function in the tumourigenic process.

Although aberrant DNA methylation was the first cancer-related epigenetic alteration reported, there are numbers of reports concerning its deregulation in chordoma. Rinner *et al.* showed 20 hyper/hypomethylated genes using AITCpG360 methylation assay; these included *C3*, *XIST*, *TACSTD2*, *FMRI*, *HIC1*, *RARB*, *DLEC1*, *KL* and *RASSF1*. Chordoma showed a characteristic DNA methylation pattern, resulting in significant genomic instability (34). These findings provide novel targets in chordoma pathogenesis, diagnosis and therapeutic options. MGMT (O6-methylguanine-DNA methyltransferase) gene promoter methylation was previously proven to be present in gliomas, acting as a

prognostic and predictive marker (35). Presence of methylated MGMT is positively linked to efficacy of temozolomide (TMZ), an anti-cancer drug. Marucci *et al.* showed that a high portion of methylated MGMT promoter was present in recurring clival chordoma, whereas its promoter was always unmethylated in clival chordoma without recurrence (35); that study helped predict clinical post-operative outcomes of chordoma. In a recent investigation, DNA methylation profiling revealed that 8819 loci (2.9%) were differentially methylated in chordoma, among which 5868 probes (66.5%) were hypomethylated and 2,951 (33.5%) were hypermethylated (36). In addition, 104 probes (104/2951) demonstrated cancer-specific hypermethylation in chordoma. These findings indicated that cancer-specific hypermethylation loci might also play a crucial role in development of chordoma, which still needs further investigation. Longoni *et al.* (37) found seven (7/13) chordoma specimens with methylated DNA correlating with transcriptional silencing of the gene. *CDKN2A* gene, located on 9p21, has been shown to be homo- or heterozygously lost in 70% of chordomas. Using methylation specific PCR, tumour suppressor proteins *CDKN2A* and *PTEN* promoter hypermethylation were evaluated using methylation specific PCR (MSP). Only one case, *CDKN2A*, showed definitive promoter methylation, indicating that promoter methylation may explain *CDKN2A* gene silencing in only a small subset of chordomas. In addition, transcriptional silencing of *PTEN* was not found to be associated with methylation status in the promoter region of the gene (37–40). Le *et al.* observed methylated DNA sequences in two and four of fifteen cases for *CDKN2A* and *PTEN* respectively (38).

Histone modification in chordoma

Histone deacetylases (HDACs) are a class of enzymes that can remove acetyl groups from histones, altering accessibility of transcription factors to gene promoter regions (41). Besides influencing histone, HDACs can affect malignant transformation by acting on non-histone proteins too. HDAC inhibitors are a novel class of anti-cancer drugs being tested in various clinical trials for various cancers (42). Vorinostat (SAHA) and Panobinostat (LBH-589) are Pan-HDAC inhibitors. Abnormal expression of HDACs, which regulate balance of histone acetylation and deacetylation, has been observed in chordoma. Using immunochemistry, Scheipl *et al.* observed expression of HDACs 2–6, with the strongest expression of HDAC6 in chordoma (43). Furthermore, Pan-HDAC inhibitors, SAHA and LBH-589, significantly promote apoptosis and change cell cycle distribution in chordoma cells *in vitro*. This study has suggested

potential use in future, for HDAC inhibitors as therapeutic options for chordoma.

MicroRNAs in chordoma

Over recent years, accumulating data have suggested that miRNAs play significant roles in pathogenesis of various cancers, including chordoma, and to date, several studies have investigated roles of miRNAs in it (44,45). As well as regulating genes involved in the cell cycle, cell proliferation, apoptosis, angiogenesis, invasion and metastasis, miRNAs play a crucial role in pathogenesis and development of chordoma (45,46).

Expression of miR-1 and miR-206 are reduced in both chordoma tissues and cell lines. In addition, Met and HDAC4 have been proven to be miR-1 targets overexpressed in chordomas (44). Met is a proto-oncogene overexpressed in many human cancers, including chordoma; restoration of miR-1 expression suppresses migratory and invasive activities of chordoma cells. These findings implicate a potential effect of miR-1 on chordoma pathogenesis, and by downregulating Met, miR-1 might act as a tumour suppressor here. Furthermore, Duan *et al.* showed that miR-1 expression levels were also associated with clinical prognosis (45). They also found that miR-1 reduced cell population growth and proliferation in chordoma cells *in vitro*. A further study has also shown that miR-1 reduced cell proliferation, as well as migratory and invasive activities of chordoma cells. In addition, Slug, over-expressed in chordoma cell lines and advanced chordoma lesions, has been identified to be the direct target of miR-1. Thus, the miR-1–Slug pathway has crucial roles in chordoma cell population growth and migration and may itself serve as a potential therapeutic target (46). Zou *et al.* have identified 29 differentially expressed miRNAs in chordomas compared to normal nucleus pulposus tissues. In addition, patients with low miR-1237-3p expression had the worse recurrence-free survival of patients, with high chordoma invasion (47).

To determine miRNA expression profiles in chordoma, Bayrak *et al.* performed miRNA arrays of chordoma samples compared to those of normal nucleus pulposus (48). Thirty up-regulated and 23 down-regulated miRNAs were identified in chordoma. Among all deregulated miRNAs, the most up-regulated included miR-140-3p and miR-148a, and most down-regulated were miR-31 and miR-222, confirmed by polymerase chain reaction. In addition, miR-31 promoted apoptosis in chordoma cells by down-regulating c-MET and radixin. Kuang *et al.* showed that miR-10a and miR-125a were reduced in chordoma compared to controls (49); miR-140-3p was up-regulated in chordomas, positively correlating with muscle invasion. In addition, over-

Table 1. Functional characterization of the deregulated miRNAs in chordoma

Name	Up- or down-regulation	Target gene	Role	Reference
miR-1	Down	Met, HDAC4, Slug	Tumour suppressor	(44–46)
miR-206	Down		Tumour suppressor	(44)
miR-1237-3p	Down		Tumour suppressor	(47)
miR-140-3p	Up		Oncogene	(7)
miR-148a	Up		Oncogene	(48)
miR-31	Down	c-MET, radixin	Tumour suppressor	(48)
miR-222	Down		Tumour suppressor	(48)
miR-10a	Down		Tumour suppressor	(49)
miR-125a	Down		Tumour suppressor	(49)
MiR-140-3p	Up		Oncogene	(50)
MiR-608	Down	EGFR, Bcl-xL	Tumour suppressor	(50)
miR-34a	Down	MET	Tumour suppressor	(50)

expression of miR-140-3p predicted poorer recurrence-free survival and served as an independent prognostic factor for chordoma patients. miR-608 and miR-34a expressions were down-regulated in human chordoma cell lines UCH1 and UCH2. Their restoration reduced cell proliferation and invasion and induced apoptosis (50). In addition, EGFR and Bcl-xL, oncogenes, were proven to be direct targets of miR-608, while MET was the direct target of miR-34a. Expression of miR-34a and miR-608 was inversely correlated to MET and EGFR expression, respectively, in chordoma cells. Furthermore, EGFR and MET activations increased cell proliferation and invasion, while pharmacological inhibition of EGFR and MET reduced proliferation and survival. These findings indicate that miR-608 and miR-34a act as tumour suppressors in chordoma by targeting these oncogenes (Table 1). Fragile histidine triad (FHIT) is a tumour suppressor, implicated in cancers of tissues arising from all three germ cell layers. Diaz *et al.* found high rates of low-FHIT protein expression in sacral (98%) and skull base chordoma (67%) while there was only 21% classic clival chordoma with chromosomal loss or gain encompassing the FHIT locus. These results imply that epigenetic mechanisms in addition to genomic instability regulate FHIT expression in chordoma (51).

Potential therapeutic methods based on epigenetic mechanisms

Management of chordoma is a clinical challenge, requiring multimodal therapy – yet there is poor overall

survival. Significant improvements have made by discovery of epigenetic involvement in this disease. However, targeting or modulating epigenetic remodelling enzymes and transcription factor can reprogram epigenetic deregulation and attenuate the malignant phenotype. For example, by use of inhibitors of DNA methyltransferases DNMTs and HDACs, epigenetically silenced genes have been re-expressed successfully *in vitro*. Traditional DNMT inhibitors along with second-generation DNMT inhibitors greatly improve efficacy of epigenetic therapies with fewer side effects. Furthermore, the US Food and Drug Administration have approved some of these compounds for elective treatment of myelodysplastic syndromes 5-azacytidine and 5-aza-20-deoxycytidine [DNMT inhibitors] and cutaneous T-cell lymphomas, suberoyl anilide hydroxamic acid [SAHA] and romidepsin, which are HDAC inhibitors (HDACi) (52–54). For example, pan-HDAC-inhibitors Vorinostat and Panobinostat (LBH-589) promote apoptosis and change cell cycle distribution in chordoma cells *in vitro* (43). Patients with chordoma will benefit from additional treatment options with epigenetic alterations. In addition, miRNAs are also expected to provide relevant targets for development of specific anti-cancer molecules that may simultaneously target several key pathways. By down-regulating Met, miRNA-1 seems to be able to act as a tumour suppressor in chordoma (46).

Acknowledgements

This work was supported by grant from the National Natural Science Foundation of China (NSFC) (Grant number: 81401847).

References

- Sciubba DM, Chi JH, Rhines LD, Gokaslan ZL (2008) Chordoma of the spinal column. *Neurosurg. Clin. N. Am.* **19**, 5–15.
- Fuchs B, Dickey ID, Yaszemski MJ, Inwards CY, Sim FH (2005) Operative management of sacral chordoma. *J. Bone Joint Surg. Am.* **87**, 2211–2216.
- Osaka S, Kodoh O, Sugita H, Osaka E, Yoshida Y, Ryu J (2006) Clinical significance of a wide excision policy for sacrococcygeal chordoma. *J. Cancer Res. Clin. Oncol.* **132**, 213–218.
- Stacchiotti S, Casali PG, Lo Vullo S, Mariani L, Palassini E, Mercuri M *et al.* (2010) Chordoma of the mobile spine and sacrum: a retrospective analysis of a series of patients surgically treated at two referral centers. *Ann. Surg. Oncol.* **17**, 211–219.
- Chambers PW, Schwinn CP (1979) Chordoma. A clinicopathologic study of metastasis. *Am. J. Clin. Pathol.* **72**, 765–776.
- Zerres K, Eggermann T (2014) [Genetics and epigenetics. Explanatory approaches for (gender-specific) mechanisms of disease development]. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* **57**, 1047–1053.

- 7 Lee JJ, Murphy GF, Lian CG (2014) Melanoma epigenetics: novel mechanisms, markers, and medicines. *Lab. Invest.* **94**, 822–838.
- 8 Deans C, Maggert KA (2015) What do you mean, “epigenetic”? *Genetics* **199**, 887–896.
- 9 Feinberg AP, Tycko B (2004) The history of cancer epigenetics. *Nat. Rev. Cancer* **4**, 143–153.
- 10 Waddington CH (2012) The epigenotype. 1942. *Int. J. Epidemiol.* **41**, 10–13.
- 11 Goldberg AD, Allis CD, Bernstein E (2007) Epigenetics: a landscape takes shape. *Cell* **128**, 635–638.
- 12 Lopez-Serra L, Esteller M (2008) Proteins that bind methylated DNA and human cancer: reading the wrong words. *Br. J. Cancer* **98**, 1881–1885.
- 13 Schubeler D (2015) Function and information content of DNA methylation. *Nature* **517**, 321–326.
- 14 Baylin SB (2005) DNA methylation and gene silencing in cancer. *Nat. Clin. Pract. Oncol.* **2**(Suppl 1), S4–S11.
- 15 Dean W, Lucifero D, Santos F (2005) DNA methylation in mammalian development and disease. *Birth Defects Res. C. Embryo Today* **75**, 98–111.
- 16 Stirzaker C, Taberlay PC, Statham AL, Clark SJ (2014) Mining cancer methylomes: prospects and challenges. *Trends Genet.* **30**, 75–84.
- 17 Robertson KD (2005) DNA methylation and human disease. *Nat. Rev. Genet.* **6**, 597–610.
- 18 Kulis M, Esteller M (2010) DNA methylation and cancer. *Adv. Genet.* **70**, 27–56.
- 19 Strahl BD, Allis CD (2000) The language of covalent histone modifications. *Nature* **403**, 41–45.
- 20 Ito K, Barnes PJ, Adcock IM (2000) Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1 β -induced histone H4 acetylation on lysines 8 and 12. *Mol. Cell. Biol.* **20**, 6891–6903.
- 21 Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F *et al.* (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev.* **18**, 1263–1271.
- 22 Shilatifard A (2006) Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu. Rev. Biochem.* **75**, 243–269.
- 23 Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. *Cell* **128**, 669–681.
- 24 Yu X, Li Z, Liu J (2015) Mirnas in primary cutaneous lymphomas. *Cell Prolif.* **48**, 271–277.
- 25 Li Z, Yu X, Shen J, Jiang Y (2015) MicroRNA dysregulation in uveal melanoma: a new player enters the game. *Oncotarget* **6**, 4562–4568.
- 26 Palanichamy JK, Rao DS (2014) MicroRNA dysregulation in cancer: towards a mechanistic understanding. *Front. Genet.* **5**, 54.
- 27 Li Z, Yu X, Wang Y, Shen J, Wu WK, Liang J *et al.* (2014) By downregulating *tiam1* expression, microRNA-329 suppresses gastric cancer invasion and growth. *Oncotarget* **6**, 17559–17569.
- 28 Wang X, Zhao J, Huang J, Tang H, Yu S, Chen Y (2012) The regulatory roles of microRNA and methylation on oncogene and tumor suppressor gene expression in pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* **425**, 51–57.
- 29 Hu Y, Zhou J, Ye F, Xiong H, Peng L, Zheng Z *et al.* (2015) Brd4 inhibitor inhibits colorectal cancer growth and metastasis. *Int. J. Mol. Sci.* **16**, 1928–1948.
- 30 Fiskus W, Sharma S, Qi J, Valenta JA, Schaub LJ, Shah B *et al.* (2014) Highly active combination of brd4 antagonist and histone deacetylase inhibitor against human acute myelogenous leukemia cells. *Mol. Cancer Ther.* **13**, 1142–1154.
- 31 Segura MF, Fontanals-Cirera B, Gaziel-Sovran A, Guijarro MV, Hanniford D, Zhang G *et al.* (2013) Brd4 sustains melanoma proliferation and represents a new target for epigenetic therapy. *Cancer Res.* **73**, 6264–6276.
- 32 Rusiecki JA, Al-Nabhani M, Tarantini L, Chen L, Baccarelli A, Al-Moundhri MS (2011) Global DNA methylation and tumor suppressor gene promoter methylation and gastric cancer risk in an omani arab population. *Epigenomics* **3**, 417–429.
- 33 Skowronski K, Andrews J, Rodenhiser DI, Coomber BL (2014) Genome-wide analysis in human colorectal cancer cells reveals ischemia-mediated expression of motility genes via DNA hypomethylation. *PLoS ONE* **9**, e103243.
- 34 Rinner B, Weinhaeusel A, Lohberger B, Froehlich EV, Pulverer W, Fischer C *et al.* (2013) Chordoma characterization of significant changes of the DNA methylation pattern. *PLoS ONE* **8**, e56609.
- 35 Marucci G, Morandi L, Mazzatenta D, Frank G, Pasquini E, Foschini MP (2014) MGMT promoter methylation status in clival chordoma. *J. Neurooncol* **118**, 271–276.
- 36 Alholle A, Brini AT, Bauer J, Gharanei S, Niada S, Slater A *et al.* (2015) Genome-wide DNA methylation profiling of recurrent and non-recurrent chordomas. *Epigenetics* **10**, 213–220.
- 37 Longoni M, Orzan F, Stroppi M, Boari N, Mortini P, Riva P (2008) Evaluation of 1p36 markers and clinical outcome in a skull base chordoma study. *Neuro Oncol* **10**, 52–60.
- 38 Le LP, Nielsen GP, Rosenberg AE, Thomas D, Batten JM, Deshpande V *et al.* (2011) Recurrent chromosomal copy number alterations in sporadic chordomas. *PLoS ONE* **6**, e18846.
- 39 Choy E, MacConaill LE, Cote GM, Le LP, Shen JK, Nielsen GP *et al.* (2014) Genotyping cancer-associated genes in chordoma identifies mutations in oncogenes and areas of chromosomal loss involving *cdkn2a*, *pten*, and *smarcb1*. *PLoS ONE* **9**, e101283.
- 40 Hallor KH, Staaf J, Jonsson G, Heidenblad M, Vult von Steyern F, Bauer HC *et al.* (2008) Frequent deletion of the *cdkn2a* locus in chordoma: analysis of chromosomal imbalances using array comparative genomic hybridisation. *Br. J. Cancer* **98**, 434–442.
- 41 Seto E, Yoshida M (2014) Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb. Perspect. Biol.* **6**, a018713.
- 42 Li Z, Zhu WG (2014) Targeting histone deacetylases for cancer therapy: from molecular mechanisms to clinical implications. *Int. J. Biol. Sci.* **10**, 757–770.
- 43 Scheipl S, Lohberger B, Rinner B, Froehlich EV, Beham A, Quehenberger F *et al.* (2013) Histone deacetylase inhibitors as potential therapeutic approaches for chordoma: an immunohistochemical and functional analysis. *J. Orthop. Res.* **31**, 1999–2005.
- 44 Duan Z, Choy E, Nielsen GP, Rosenberg A, Iafrate J, Yang C *et al.* (2010) Differential expression of microRNA (mirna) in chordoma reveals a role for mirna-1 in met expression. *J. Orthop. Res.* **28**, 746–752.
- 45 Duan Z, Shen J, Yang X, Yang P, Osaka E, Choy E *et al.* (2014) Prognostic significance of mirna-1 (mir-1) expression in patients with chordoma. *J. Orthop. Res.* **32**, 695–701.
- 46 Osaka E, Yang X, Shen JK, Yang P, Feng Y, Mankin HJ *et al.* (2014) MicroRNA-1 (mir-1) inhibits chordoma cell migration and invasion by targeting slug. *J. Orthop. Res.* **32**, 1075–1082.
- 47 Zou MX, Huang W, Wang XB, Li J, Lv GH, Wang B *et al.* (2015) Reduced expression of mirna-1237-3p associated with poor survival of spinal chordoma patients. *Eur. Spine J.* **24**, 1738–1746.
- 48 Bayrak OF, Gulluoglu S, Aydemir E, Ture U, Acar H, Atalay B *et al.* (2013) MicroRNA expression profiling reveals the potential function of microRNA-31 in chordomas. *J. Neurooncol.* **115**, 143–151.

- 49 Kuang L, Lv G, Wang B, Li L, Dai Y, Li Y (2015) Overexpression of adenosine deaminase acting on rna 1 in chordoma tissues is associated with chordoma pathogenesis by reducing mir125a and mir10a expression. *Mol Med Rep* **12**, 93–98.
- 50 Zhang Y, Schiff D, Park D, Abounader R (2014) Microrna-608 and microrna-34a regulate chordoma malignancy by targeting egfr, bcl-xl and met. *PLoS ONE* **9**, e91546.
- 51 Diaz RJ, Guduk M, Romagnuolo R, Smith CA, Northcott P, Shih D *et al.* (2012) High-resolution whole-genome analysis of skull base chordomas implicates fh1t loss in chordoma pathogenesis. *Neoplasia* **14**, 788–798.
- 52 Atallah E, Garcia-Manero G (2007) Use of hypomethylating agents in myelodysplastic syndromes. *Clin. Adv. Hematol. Oncol.* **5**, 544–552.
- 53 Momparler RL, Cote S, Momparler LF (2013) Epigenetic action of decitabine (5-aza-2'-deoxycytidine) is more effective against acute myeloid leukemia than cytotoxic action of cytarabine (ara-c). *Leuk. Res.* **37**, 980–984.
- 54 Thaler F (2012) Current trends in the development of histone deacetylase inhibitors: a review of recent patent applications. *Pharm. Pat. Anal.* **1**, 75–90.