INTERNATIONAL JOURNAL OF Experimental Pathology

Int. J. Exp. Path. (2010), 91, 387–393

REVIEW ARTICLE

Therapeutic molecular targets in human chondrosarcoma

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INTERNATIONAL JOURNAL OF EXPERIMENTAL PATHOLOGY

doi: 10.1111/j.1365-2613.2010.00749.x

Received for publication: 12 August 2010
Accepted for publication: 17 October 2010

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Summary

Chondrosarcomas are malignant cartilage tumours. They are poorly responsive to chemotherapy and radiotherapy. Treatment is usually limited to surgical resection; however, survival of patients with high-grade chondrosarcoma is poor, even with wide surgical resection. Induction of apoptosis in chondrosarcoma cells, either directly or by enhancement of the response to chemotherapeutic drugs and radiation, may be a route by which outcome can be improved. In this article, we review potential molecular targets that regulate chondrocyte apoptosis and discuss the experimental evidence for their utility.

Keywords
apoptosis, cartilage, chemotherapy, chondrocytes, chondrosarcoma, radiation

Chondrocytes

Chondrocytes are long-lived, slowly cycling, specialized cells that secrete the extracellular matrix (ECM) that forms cartilage. The matrix they secrete separates them from each other so that they only receive cell membrane contact signals from ECM components. Cartilage has little or no blood or lymphatic supply. Thus, whilst they are exquisitely sensitive to mechanical signalling, chondrocytes can only receive and send chemical messages that can diffuse through the surrounding matrix. Like all cell types, chondrocytes can undergo malignant transformation but their unique environment means that chondrosarcomas pose particular problems for treatment and clinical management.

Chondrosarcomas

Chondrosarcomas are a heterogeneous group of malignant tumours showing hyaline cartilage differentiation. The majority of chondrosarcomas are slow growing and rarely metastasize (Gelderblom *et al.* 2008). They are primarily tumours of bone and are predominantly found in adults where they are the third most common malignant bone tumour. Prognosis depends largely on the histological grade. Low-grade lesions are matrix rich with low cellularity. Higher-grade tumours show increased cellularity, mitotic activity and cellular pleomorphism (Evans *et al.* 1977). Conventional chondrosarcomas account for the vast majority and approximately 90% of these are low- to intermediate-grade tumours characterized by indolent clinical behaviour and low metastatic potential. Most of the remainder are high-grade lesions, which commonly metastasize. Chondrosarcomas arise as primary tumours in previously normal bone or as secondary tumours in benign precursor lesions such as osteochondroma or enchondromas. Patients with Ollier's disease (multiple enchondromatosis) and hereditary multiple exostosis are particularly at risk of developing secondary chondrosarcomas. Dedifferentiated chondrosarcoma is a distinct variety of chondrosarcoma in which a well-differentiated low-grade cartilage tumour is juxtaposed to a high-grade non-cartilaginous sarcoma. These are aggressive lesions with poor prognosis.

Current treatment

The clinical management of chondrosarcoma remains a challenging problem. Disease-free survival and overall survival of patients with chondrosarcoma have not shown any significant improvement over the last few decades. Surgical resection remains the treatment of choice for the majority of chondrosarcomas, and complete resection of low- to intermediate-grade tumours is often curative. However, there are a significant number of chondrosarcomas, especially those arising in the pelvis or the spine, that are difficult to remove with a wide surgical margin (Bergh *et al.* 2001; Bruns *et al.* 2001). In these instances, local recurrence is almost inevitable and becomes a serious clinical problem.

As low-grade chondrosarcomas possess a low percentage of dividing cells within a poorly vascular matrix, they are relatively radiation and chemo-resistant (Gelderblom *et al.* 2008) and chemotherapy and radiotherapy do not improve the treatment outcome. (Johnson *et al.* 1986; Lee *et al.* 1999). Similarly, there is little role for chemotherapy or radiotherapy in management of patients with high-grade chondrosarcomas or with metastatic disease where outcome remains poor. There are reasons why chondrosarcomas are chemoresistant; tissue access of chemotherapeutic drugs is more difficult in chondrosarcomas than in vascularized cancers as the drugs have to diffuse over a long distance to reach tumour cells; chondrosarcomas have high expression of the multidrug resistance–associated protein P-glycoprotein, a product of the ABCB1 gene, (Terek *et al.* 1998) so that any drug effect is limited; in addition, chondrosarcomas grow relatively slowly, whilst most chemotherapeutic drugs act on rapidly dividing cells (Staals *et al.* 2006; Gelderblom *et al.* 2008). The mechanism of radiation resistance in chondrosarcomas is increasingly understood. Radiation resistance may be related to local tissue hypoxia as has been described in other tumours, the relative low levels of oxygen preventing the formation and propagation of radiation-induced reactive oxygen species that cause single and double strand DNA damage (Dunst *et al.* 2003). Expression of HIF-1α by chondrosarcomas in association with increased levels of Bcl-2 can contribute to both radio resistance and chemoresistance (Kubo *et al.* 2008). $p16^{ink4a}$, a tumour suppressor protein, also appears to have a role in radiation resistance of chondrosarcomas (Moussavi-Harami *et al.* 2006).

Recently, there has been increased focus on developing anti-cancer therapies aimed at controlling the function and expression of molecules involved in cell death/survival and proliferation pathways. Targeting apoptosis pathways and inducing cell death directly or by enhancing the response of malignant cells to chemotherapeutic agents and radiation therapy have the potential to improve outcomes in those patients with chondrosarcomas who have inoperable, recurrent and metastatic disease. For instance, expression of genes that control cell death and survival, such as Bcl-2 family members, is well documented in chondrosarcomas (Daugaard *et al.* 2009). Short-interfering (si) RNA targeting Bcl-2 family members reverse chemoresistance and increase radiation sensitivity in P-glycoprotein expressing chondrosarcoma cell lines, suggesting that Bcl-2 family gene silencing may be a therapeutic target for chondrosarcomas (Kim *et al.* 2009).

In this review, we will discuss what is known of the factors that regulate chondrocyte proliferation and cell death and overview related therapeutic targets that have the potential to improve treatment of patients with chondrosarcoma.

Regulation of chondrocyte proliferation and apoptosis

Much of what is known about the regulation of chondrocyte proliferation and apoptosis has come from studies of bone development and growth and understanding of cellular abnormalities in osteoarthritic articular cartilage. Chondrocyte proliferative activity is most evident in developing cartilage and in the proliferative zone of the growth plate. In the mature skeleton of adults, normal chondrocyte proliferation and cartilage growth is limited. In chondrosarcomas, the normal physiological balance between cell proliferation and apoptosis is lost. Both proliferation and cell death may increase but an overall increase in the rate of cell proliferation over apoptosis leads to excess proliferation and growth of neoplastic cells.

Chondrocyte proliferation

In the growth plate, chondrocyte proliferation and differentiation are regulated by parathyroid hormone–related peptide (PTHrP) and Indian hedgehog (Ihh). Parathyroid hormone–related peptide produced by periarticular chondrocytes and perichondrial cells acts on chondrocytes expressing PTH/PTHr receptors inhibiting apoptosis, maintaining proliferation and delaying differentiation into prehypertrophic and subsequently hypertrophic chondrocytes (Burdan *et al.* 2009). PTHrP activates the Gs family of G proteins and through cAMP and PKA decreases the expression of p57 a cyclin-dependent kinase inhibitor (Kronenberg 2006). Cyclins are involved in the regulation of chondrocyte proliferation in the growth plate with cyclin-D1 showing restriction to the proliferative zone. Cyclins complexed with cyclin-dependent kinases can initiate a signal cascade involving pRB and E2F transcription factors leading to cell cycle progression. Bone morphogenetic proteins (BMPs) also support chondrocyte proliferation by blocking the inhibitory effect of FGF signalling via FGFR3 (Oji *et al.* 2007). Ihh in turn is a key regulator of chondrogenesis by regulating PTHrP production. Under the actions of PTHrP, cartilage grows and chondrocytes become removed from the source of PThrP. As the effect of PTHrP decreases, the cells stop proliferating, begin to secrete Ihh and maturation and differentiation pathways are activated. Release of Ihh by the now non-proliferating chondrocytes stimulates the synthesis of PTHrP by periarticular chondrocytes and perichondrial cells via a feedback loop involving the hedgehog-activated transcription factor Gli1. Ihh may also increase chondrocyte proliferation independently of PThrP (Long *et al.* 2001).

Chondrocyte apoptosis

There are two alternative pathways, the extrinsic and intrinsic pathways for the initiation of apoptosis. Ultimately, both pathways lead to the activation of caspases that cleave cellular substrates and induce the biochemical and the morphological changes characteristic of apoptosis (see e.g. reviews by Moffitt *et al.* 2010; and Falschlehner *et al.* 2009).

The extrinsic pathway is mediated by the death receptors DR4/DR5 and CD95/Fas, which are activated by tumour necrosis factor-alpha (TNFα), FasL or tumour necrosis factor–related apoptosis-inducing ligand (TRAIL). Following receptor–ligand interactions, there is assembly of the death signalling complex (DISC) that recruits initiator caspases, including caspase-8 with the activation of executioner proteases such as caspase-3. These death receptors and ligands have been demonstrated in human osteoarthritic cartilage with in vitro studies confirming that activation of the receptors by the appropriate ligand induces apoptosis (Lee *et al.* 2004).

The intrinsic pathway, activated by a variety of cellular stresses such as DNA damage, mechanical injury, hypoxia, loss of growth factor and matrix survival signals also appear to be important in chondrocyte apoptosis. Central to the intrinsic pathway are changes in the ratio of pro-apoptotic and anti-apoptotic members of the BCL-2 family (Brenner & Mak 2009). These function as either pro-apoptotic regulators such as Bax, Bad or PUMA (p53 upregulated modulator of apoptosis) or as anti-apoptotic proteins including Bcl-2 and Bcl-xl. BCL-2 family members act by promoting or blocking changes in mitochondrial membrane permeability. Increased mitochondrial membrane permeability results in the release of pro-apoptotic factors, including cytochrome c and activation of caspase 9 and caspase 3, which leads to apoptosis. Whether a chondrocyte commits to death or survival under stress will in part depend on the ratio of pro-apoptotic proteins to anti-apoptotic proteins.

Chondrocytes may also undergo apoptosis as part of the unfolded protein response (UPR). Under stress conditions such as hypoglycaemia or hypoxia, proteins in the endoplasmic reticulum (ER) may not undergo folding or glycosylation and accumulate as unfolded proteins. These bind to a chaperone protein called glucose-regulated protein 78 that leads to activation of the UPR. When this adaptive response is prolonged rather than alleviating cell stress, the UPR leads to apoptosis through activation of caspase 12. Endoplasmic reticulum stress–induced apoptosis of chondrocytes appears to be important in pseudochondroplasia (Hashimoto *et al.* 2003).

Regulators of cell death as therapeutic targets in chondrosarcoma

In conditions such as osteoarthritis where increased chondrocytes death is recognized as being a contributing factor to disease pathogenesis, the aim is to inhibit chondrocytes apoptosis. The main cellular targets are illustrated in Figure 1. In chondrosarcoma, where chondrocyte proliferation is increased and cell death is relatively reduced, the converse holds. Targeting the apoptosis pathways either directly to enhance tumour cell death or indirectly increase the efficacy of standard treatments should result in decreased tumour growth (See Table 1).

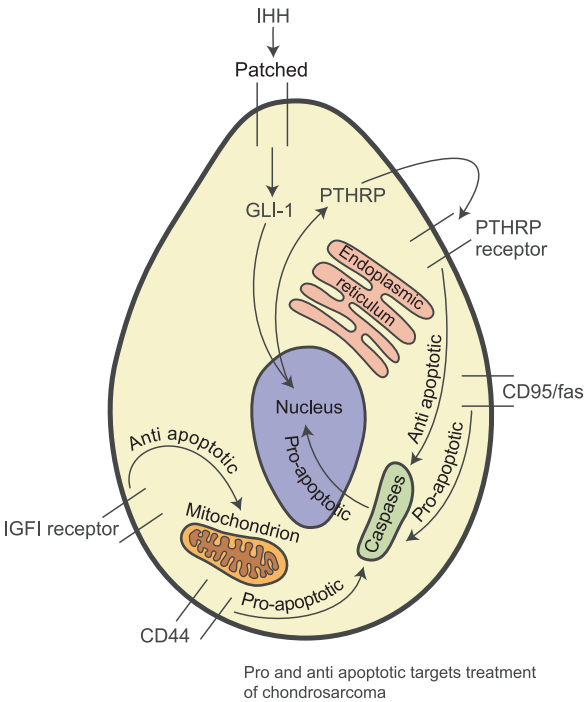

Figure 1 This figure illustrates the key potential targets for therapy aimed at inducing apoptosis in chondrosarcoma cells.

Table 1 Therapeutic targets and active agents that increase apoptosis in chondrosarcomas

Mechanism	Therapeutic agents
Modulation of apoptosis signal cascades	2ME
	PEDF
	EGCG
	PPARγ ligands
Phloroglucinol derivatives	BFPP
Blockade of cell receptor function	Monoclonal antibodies
	Anti-PTHrP
	Anti-CD44
Enhancers of Radiotherapy and Chemotherapy	rhPDCD5
	anti-apoptotic siRNAs
Others	HDAC inhibitors
	Bisphosphonates
	Collagen propeptides

2ME, 2-Methoxyestardiol; PEDF, pigment epithelium–derived factor; EGCG, Epigallocatechin-3-gallate; PPARγ, Peroxisome proliferator-activated receptor gamma; BFPP, 2, 4-bis (fluorophenylacetyl) phloroglucinol; PTHrP, parathyroid hormone–related peptide; rhPDCD5, Recombinant human programmed cell death; HDAC, Histone deacetylase.

Modulators of apoptosis pathways

Methoxyestradiol (2ME). Methoxyestradiol (2ME) a metabolite of 17beta-estradiol is a microtubule inhibitor that is a known anti-angiogenic agent with anti-proliferative and cytotoxic activity in some solid tumours (Sutherland *et al.* 2007). Although believed to have activity only against actively dividing cells, 2ME was shown to induce apoptosis in non-proliferating, differentiated hypertrophic chondrocytes of the rat growth plate (Sibonga *et al.* 2002). Subsequently, effects of 2ME on chondrosarcoma cells have been studied in vitro (Fong *et al.* 2007). In the grade 2 chondrosarcoma cell line, JJ012, 2ME increases apoptosis in addition to inducing cell cycle arrest. The increase in apoptosis was a result of increasing Bax, caspase-3 and cytochrome C protein expression levels whilst decreasing p53 expression. As Bcl-2 expression was not significantly changed, the Bax/Bcl-2 ratio was pro-apoptotic.

Pigment epithelium–derived factor (PEDF). Pigment epithelium–derived factor (PEDF), a 50 kDa glycoprotein, is a member of the serpin (serine protease inhibitor) family. It was initially identified as an anti-angiogenic agent produced by retinal epithelium. In addition to inhibiting vascularization, PEDF has anti-tumour activity by inducing apoptosis via the FAS/FASL pathway. At least part of PEDF pro-apoptotic activity is by downregulation of the decoy c-FLIP (Zaichuk *et al.* 2004) that inhibits the extrinsic apoptosis pathway by competing with caspase-8 for FADD binding. *In vitro* PEDF decreases chondrosarcoma proliferation and increases apoptosis through stimulation of the extrinsic apoptosis pathway. Treatment of JJ102 cells with PEDF increased expression of Fas, caspase 6 and caspase 3 although there was no change in FasL expression. PEDF also increased adhesion to type I collagen and decreased invasion capacity of the chondrosarcoma cell line.

Epigallocatechin-3-gallate (EGCG). Epigallocatechin-3-gallate (EGCG) is the ester of epigallocatechin and gallic acid. It is a naturally produced compound and the major polyphenol in green tea. Epigallocatechin-3-gallate is an anti-oxidant and is believed to have a number of health benefits including anti-inflammatory activity, enhancement of humoral and cell-mediated immunity and decreasing the risk of certain cancers (Butt & Sultan 2009; Clement 2009). Epigallocatechin-3-gallate can inhibit carcinogenesis and the growth of established cancers at various organ sites (Khan & Mukhtar 2007). In normal and osteoarthritic cartilage, EGCG has anti-inflammatory effects. Studies on human chondrosarcoma cell lines suggest that there may also be beneficial effects against cartilage tumours. Observations that treatment of the human chondrosarcoma cell line HTB-94 with EGCG reduced cell viability and induced apoptosis (Islam *et al.* 2000) were recently extended by Tang *et al.* (2010) who additionally showed effects on SW1353 and CRL-7891 chondrosarcoma cell lines. In these studies, EGCG inhibited the Ihh pathway by downregulating PTCH and Gli-1 levels and induced apoptosis. Caspase-3 was unchanged, whilst levels of Bcl-2 were significantly decreased and the levels of Bax were significantly increased.

Peroxisome proliferator-activated receptor gamma (PPARγ) ligands. Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor that forms heterodimers with retinoid × receptors to regulate gene transcription. PPARγ is expressed by many tumours and is involved in cell proliferation and apoptosis (Kersten *et al.* 2000). Peroxisome proliferator-activated receptor gamma is frequently expressed by conventional chondrosarcoma and is present in chondrosarcoma cell lines (Nishida *et al.* 2008). Treatment of OUMS-27 cells, established from a grade III human chondrosarcomas, in vitro with the synthetic PPARγ ligand pioglitazone and 15d-PGJ2, the most potent endogenous ligand for PPARγ, induces apoptosis (Nishida *et al.* 2002). This effect appears to be mediated through modification of the intrinsic apoptosis pathway with PPARγ ligands inducing downregulation of Bcl-xl and increased expression of Bax (Nishida *et al.* 2008). 15d-PGJ2 also induced expression of the CDK inhibitor p21 protein in human chondrosarcoma cells, resulting in p53-independent cell cycle arrest.

Phloroglucinol derivatives

Phloroglucinols occur naturally in certain plant species including fruit trees. These have the potential to induce apoptosis in chondrosarcoma by triggering ER stress. Phloroglucinols are known to have anti-mitotic and anti-tumour effects in a number of human cancer cell lines (Quiney *et al.* 2006). A new phloroglucinol derivative 2, 4-bis (fluorophenylacetyl) phloroglucinol (BFPP) has recently been shown to pro-apoptotic activity on two chondrosarcoma cell lines (SW1353 and JJ012) but not on primary articular chondrocytes (Liu *et al.* 2010). 2, 4-bis (fluorophenylacetyl) phloroglucinol induced apoptosis through stimulation of ER stress as indicated by elevated cytosolic calcium levels, increased expression of GRP78 and activation of caspase 12.

Monoclonal antibody therapy

Monoclonal antibody therapy is increasingly used in the treatment of neoplastic and autoimmune diseases. Antibodies against antigens expressed by tumour cells can be used to deliver radioactive and drug-activating enzymes to tumour cells allowing more targeted effects. Antibodies can also be used to block growth factor signalling and cell matrix interactions. One of the major problems with monoclonal antibody therapy in the treatment of chondrosarcomas is delivery as they are poorly vascular and embedded in a proteoglycan rich matrix. As such, most studies to date have been laboratory based. In vitro studies have demonstrated that monoclonal antibodies against anti-PTHrP may be beneficial in chondrosarcoma (Iguchi *et al.* 2001). Parathyroid

hormone–related peptide increases the expression of Bcl-2. This is inhibited by anti-PTHrP antibodies resulting in accelerated cell differentiation and apoptosis and increased expression of Bax (Miyaji *et al.* 2003). Antibodies to CD44, a receptor for hyaluronan highly expressed by chondrocytes, activate the extrinsic apoptotic pathway of the SW1353 chondrosarcoma cell line (Yoshida *et al.* 2008). This appears to be by sequestration of CD95/FAS and prevention of its trimerizaton. A phase I study with antibodies against DR5 that activate the extrinsic apoptosis pathway showed a minor response in a patient with chondrosarcoma indicating the value of further work in this field (Herbst *et al.* 2010).

Enhancers of radiotherapy and chemotherapy

A variety of strategies are being developed to help overcome the limited effectiveness of current radiotherapy and chemotherapy on chondrosarcomas. Programmed cell death 5 (PDCD5), also known as TF-1 cell apoptosis-related gene 19 (TFAR19), accelerates apoptosis in response to chemotherapeutic treatment (Wang *et al.* 2004). Recombinant human PDCD5 (rhPDCD5) sensitizes chondrosarcoma cells to cisplatin-based chemotherapy in vitro and in vivo (Chen *et al.* 2010). rhPDCD5 appears to act through the intrinsic mitochondrial pathway, increasing expression of Bax, decreasing Bcl-2 expression and stimulating release of cytochrome c from mitochondria. As chondrosarcomas express significantly higher levels of anti-apoptotic proteins (Bcl-xL, Bcl-2 and XIAP) than normal chondrocytes, the effect of knockdown of anti-apoptotic genes in chondrosarcoma cells with small-interfering RNAs (siRNAs) has been investigated (Kim *et al.* 2007). These studies demonstrate that silencing Bcl-2, Bcl-xL or XIAP genes results in increased radiosensitivity.

Other targets

In addition to those molecules that increase chondrocyte death, molecular targets that will decrease chondrocyte proliferation, increase differentiation or prevent invasion and spread are being identified from gains in the knowledge of the molecular pathology of chondrosarcomas (Bovee *et al.* 2010). Histone deacetylase (HDAC) inhibitors represent a novel class of anti-neoplastic agents (Furumatsu *et al.* 2005; Garcia-Manero & Issa 2005) that cause accumulation of cells within the sub-G_1 fraction of the cell cycle and promote chondrocytic differentiation in chondrosarcoma cell lines in vitro and in vivo studies (Sakimura *et al.* 2007; Yamamoto *et al.* 2008). Bisphosphonates, recognized as potent inhibitors of osteoclast-mediated bone resorption, are widely used to treat osteoporosis, Paget's disease, and hypercalcaemia associated with cancer appears also to have direct effects on chondrocytes. Treatment of the chondrosarcoma cell line SW1353 with a third-generation nitrogen-containing bisphosphonate (minodronate) increases apoptosis (Kubo *et al.* 2006). Alendronate reduces MMP-2 production and inhibits the invasion of chondrosarcoma cells (Lai *et al.* 2007), effects that are associated with decreased proliferation and increased apoptosis (Susa *et al.*2009). Interactions of chondrocytes with intact matrix or matrix fragments via integrins regulate cell proliferation, differentiation and invasion and are possible therapeutic targets in chondrosarcomas. The NH(2)-propeptide of the cartilage-characteristic collagen, type IIB, PIIBNP, induces death in chondrosarcoma cells through adhesion to Vβ3 and Vβ5 integrins (Wang *et al.* 2010). In contrast, the C-propeptides of procollagens I alpha1 and II (PC1CP and PC2CP) acting via beta-1 integrins promote angiogenesis and tumour progression or apoptosis respectively (Vincourt *et al.* 2010). Other potential therapeutic targets include Ihh, PTHrP, oestrogen, IGFR, PDGFRB, Src, COX-2 and chemokine receptors (Bovee *et al.* 2010).

Conclusions

Chondrosarcomas are difficult to treat. Recent advances in the understanding of the molecular events that regulate death and survival in normal and neoplastic cartilage have highlighted new targets for the treatment of chondrosarcoma. New therapies may act to directly increase chondrocyte apoptosis, enhance the effects of conventional therapies or stimulate promotion of cell differentiation. Most studies to date have been undertaken in vitro. In vivo experiments raise hope that these studies may be translated into the clinic for management of patients with chondrosarcoma. A combination of targeted therapies will most likely be the best approach to tackle the multidrug and radiation resistance mechanisms of human chondrosarcoma. These may include 'personalized' medicines based on information from genetic and proteomic profiling of individual tumours on biopsy or resection. The major problem of specifically targeting these new therapies in sufficient concentrations to a tumour that is notorious for its low vascularity however needs to be overcome. Recombinant adeno-associated virus vectors may have some utility in delivery of agents to chondrosarcomas allowing modulation of chondrocyte function (Cucchiarini *et al.* 2009). Increasing chondrosarcoma vascularity by enhancing cartilage angiogenesis will facilitate drug delivery to tumours and may be possible through inhibition of anti-angiogenic factors such as chondromodulin or stimulating VEGF production.

Acknowledgements

NJ is funded by a Medical Research Council studentship.

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