HubLE I MY MENTOR AND I **Perspective**

The role of IL-34 in the pathogenesis of osteosarcoma

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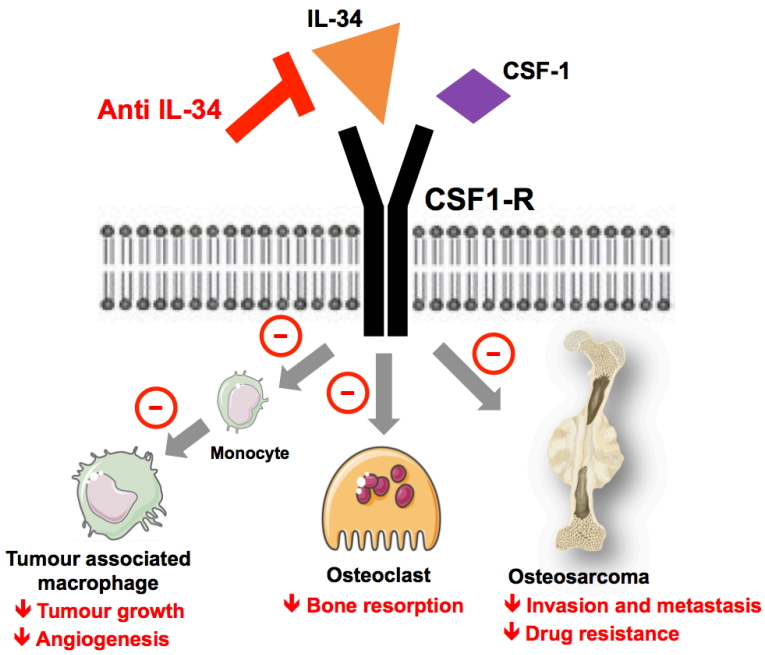
GRAPHICAL ABSTRACT



Kristina Schiavone is a PhD student at the department of Oncology and Metabolism at the University of Sheffield with an MSc in Molecular Medicine. Her research is directed at understanding the biological functions of the cytokine Interleukin (IL)-34 and its contribution to the pathogenesis of osteosarcoma. She hopes that her study will lead to the improvement of current therapies and development of individualised treatments for osteosarcoma patients.

Professor Dominique Heymann is head of the Inserm European Associated Laboratory "Sarcoma Research Unit", at the University of Nantes (France) and the University of Sheffield (UK). His main research topics are the pathogenesis of bone sarcomas, with particular interest in the dialog of bone microenvironment and tumour cells. His research aims to improve the knowledge

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on sarcomas to develop new therapeutic approaches.

ABSTRACT

Osteosarcoma (OS) is the most common malignant primary bone tumour occurring in children and adolescents. Owing to an aggressive local growth pattern, and a high predisposition to metastasize this disease has an unfavourable prognosis and is one of the leading causes of paediatric cancer associated death. Overall survival of OS patients has remained unchanged over the past 30 years highlighting the need for a better characterization of the disease and new therapeutic strategies. A key modulator in the pathogenesis of OS is the tumour niche. This is a specialised microenvironment that promotes the emergence of tumour initiating cells and provides favourable conditions for their growth and development. IL-34 is a novel cytokine with roles in bone development and the biology of bone sarcomas. IL-34 shares a common receptor with the macrophage colony stimulating factor (M-CSF), and both IL-34 and M-CSF signal through the macrophage colony stimulating factor receptor (MCSF-R). Both mediate the biology of mononuclear phagocytic cells and polarize macrophages into immunosuppressive M2 type. In osteosarcoma, IL-34 has been identified as promoting progression by increasing the tissue vasculature and stimulating the recruitment of macrophages. Consequently, IL-34 appears as a pro-metastatic regulator in osteosarcoma. Understanding the key roles of IL-34 in bone development and in the pathogenesis of OS by targeting the M-CSF/IL-34/M-CSFR triad, represents promising therapeutic approaches for OS.

INTRODUCTION

Malignant bone tumours are grouped into two categories: sarcomas, commonly referred to as malignant primary bone tumours, and carcinomas known also as secondary tumours or bone metastases. Sarcomas and carcinomas differ in the origination of their tissues. Sarcomas develop from mesodermal tissue such as bone or muscle, while carcinomas originate from epithelial tissue (endodermal or ectodermal origin) such as the lining of the breast, colon or prostate. Carcinomas do not originate from bone, but they can metastasize to bone through the lymph nodes or the blood.

Sarcomas are further subdivided into two major groups: soft tissue sarcomas and bone sarcomas. Soft tissue sarcomas develop from cells that surround, support, and protect extremities and organs of the body including fat, muscle, connective tissue, nerves and blood vessels. Bone sarcomas (primary bone tumours) originate from bone and can be of benign or malignant form. The three main types of malignant bone sarcomas are: Osteosarcoma (OS), Ewing sarcoma and chondrosarcoma[1]. While chondrosarcoma is characterized by a high risk of local recurrence and metastasis according its grading, OS and Ewing sarcoma are associated with the development of metastases to the bone, or more frequently to the lungs[2].

Primary malignant bone tumours are considerably rare and account for only 0.2% of all malignant tumours. They pre-dominate in children and teenagers, while they increase from middle age for soft tissue sarcomas. Between 1979 and 2007, around 380 people were diagnosed with bone sarcomas yearly in England. This number is however increasing as the population ages[3]. In children aged up to 14 years, almost 55 bone sarcomas are diagnosed yearly, and these account for 4% of all malignancies observed. The 5-year survival rate for patients with localized forms of sarcomas are 70-75%. Survival rates becomes around 30% for metastatic sarcomas (specifically lung metastases), and this data has not changed over the past 40 years[4].

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Osteosarcoma: Epidemiology

OS is a rare malignancy with an incidence of one to three cases per million annually[5]. It exhibits a bimodal age distribution, primarily affecting children and young people at a peak incidence of around 18 years. The second peak incidence is seen in older adults following radiotherapy or in association with Paget disease. OS is the most prevalent form of primary malignant bone tumours, describing around 56% of bone cancers in children[6]. Prevalence is higher in males than in females (male to female ratio of 1.5:1). This difference may be related to the skeletal growth period, which is longer in males than females. However it peaks earlier in females (12 years for females versus 16 years for males), due to the relatively earlier growth spurt experienced by girls[2].

Characteristics

Conventional OS is characterized as a high-grade malignant tumour composed of spindle-shaped mesenchymal cells that produce an osteoid matrix (Marina *et al.,* 2004). The current World Health Organisation (WHO) identifies three major subtypes: osteoblastic, chondroblastic and fibroblastis[5]. This classification is based on morphology and organisation of the tumour cell, including components of the extracellular matrix. Apart from these subtypes, the WHO classification describes two additional histological variants, small cell and telangiectatic OS. Telangiectatic OS is constituted of blood filled cystic spaces separated by thin septa, while small cell osteosarcoma is characterized by sheets of round cells that produce an osteoid matrix which is often confused with Ewing sarcoma[2].

OS generally develops within the metaphysis of long bones, in particular the distal femur, the proximal humerus and proximal tibia, since these regions undergo rapid growth phases. Sixty percent of cases originate in the knee, however they can also occur in the axial skeleton (<10% of cases in pediatric age group), most commonly the pelvis. Around 25% of patients present with detectable metastatic disease after 36 months from diagnosis. The most frequent site for metastases is the lungs, but they can also develop in other bones and soft tissues sites[2].

Pathogenesis

The origin of OS remains enigmatic in part due to their rarity. They are genetically unstable tumours characterized by multiple chromosomal translocations, amplifications and deletions. Recently, human sarcomas have been found to contain fusion genes associated with cellular motility, and highlight the high genetic instability characteristic of OS. Transcriptome sequencing identified two recurrent fusion genes, LRP1-SNRNP25 and KCNMB4-CCND3. These genes are known to be associated with cancer progression and where seen to promote OS cell migration and invasion[7].

Over the past two decades genome wide association studies led to progress in understanding the genetic origins of OS. A number of alterations and inactivating mutations have been found to play a role in initiating tumour development. These include mutations in the tumour suppressor genes *Tp53* and retinoblastoma (*RB1*), mutations in *c-MYC* and *RECQL4* oncogenes, and down-regulation of the Wnt signaling pathway[8]. These mutations lead to specific and rare syndromes with a predisposition to developing OS: inherited syndromes including hereditary bilateral retinoblastoma (mutation of *RB1* gene), Li–Fraumeni syndrome (germline mutations of the *p53* tumor suppressor gene), Bloom syndrome (mutation of *BLM* gene coding for a DNA helicase), Rothmund–Thomson syndrome (mutation of the RECQL4 gene encoding a DNA helicase). OS may also

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develop in association with multiple exostoses and Paget's disease. However, the majority of OS occur without any familial predisposition, and the number of cases associated with germline mutations is approximately 3%[9]. It should be noted that these data were drawn from analysis of small cohorts of tumour samples, and that none of the mutations are recurrent or specific therefore their use in diagnostics is limited[2].

A number of studies have reported difficulties in differentiating low-grade OS from benign lesions, as both present with similar radiographic appearances. Low-grade OS constitutes between 5–7% of all osteosarcomas, and is subdivided into two subgroups subject to its location to the bone cortex; parosteal and low-grade central OS. Inability to correctly diagnose these lesions may lead to inappropriate treatment. Low-grade OS is characterised by supernumerary ring chromosomes encompassing the amplification of chromosome 12q13–15, in conjunction with cyclin dependent kinase 4 (CDK4) and murine double-minute type 2 (MDM2) gene region. The incidence of these amplifications is more prevalent in low grade OS rather than high-grade classical OS and thus MDM2 and CDK4 immunohistochemistry is the current technique used to differentiate between low grade OS and benign fibrous and fibro-osseous lesions, especially in patients with atypical radio-clinical presentation and/or limited biopsy samples[10].

Although several studies have been performed to determine the events leading to the development of OS, its pathogenesis still remains unclear. Rather than looking at the genetic origin of osteosarcoma, its pathogenesis can be viewed in terms of its cellular origin, and how oncogenic events in its precursor cells lead to malignant transformation and initiation of the tumour. Within tumour sites, the local environment then helps it to survive and proliferate thus contributing further to the survival and propagation of the tumour.

Conventional Therapy

The current therapeutic approach for treating OS was established by Rosen *et al* in the late 70's. Following staging investigations and a diagnostic biopsy, initial treatment consists of intensive polychemotherapy with combinations of cisplatin, doxorubicin, methotrexate and ifosfamide. These drugs are administered prior to radical en-bloc tumour resection[11]. The resected tumour is then scored on the percentage number of residual viable tumour cells according to the Huvos scale (grade I > 50%, grade II from 11-50%, grade III from 1-0%, grade IV: no detectable viable cancer cells)[12]. In practice, patients are divided into good responders if <10% of tumour cells are found to be viable, and poor responders if >10% of viable tumour cells are present. A recent international EURAMOS-1 study determined the effect on patient survival by altering post-operative chemotherapy based on this histological response. Poor responders were randomized between continuing MAP (methotrexate, doxorubicin and cisplatin), and MAPIE (addition of ifosfamide and etoposide). Results showed that there was no significant difference in the outcome between the regimens of drugs administered and therefore post-operative chemotherapy is not adaptive according to response[13]. Radiotherapy is also administered when surgery is not possible (such as neck, head, spine), or when resection margins are considered as inadequate[14].

The Microenvironment In Osteosarcoma

Several other theories have been proposed to explain the development of cancer cells in bone. One such is based on the 'seed and soil' theory initially proposed

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by Stephen Paget in the late 19th century and focuses on OS and its local environment[15]. When tumour cells invade bone, a disruption in the balance between bone resorption and bone formation occurs, creating an inflammatory-like environment that promotes growth of tumour cells. A cycle is established between the tumour cells and their microenvironment, where the functional equilibrium between osteoblasts and osteoclasts is deregulated. During bone remodeling, osteoblasts deposit new bone tissue while osteoclasts resorb bone tissues. OS cells de-regulate the microenvironment by activating osteoclast differentiation and resorption, which in turn stimulate tumour growth by releasing proliferative factors stored in the extracellular matrix[16]. This leads to the development of the 'bone niche', in which the bone microenvironment promotes the progression of cancer initiating cells and provides the right conditions for their survival and development. Cancer initiating cells are defined as cells with self-renewal ability, tumour-initiating capacity, and ability to give rise to more differentiated progeny[17]. This not only occurs in primary malignant sarcomas, but also during the development of secondary bone metastases.

The niche is a highly complex environment and is not only restricted to bone related cells. Other types of cells including endothelial cells and macrophages are also present. These set up niches of their own, a 'vascular niche' and an 'immune niche', which contribute to the tumour microenvironment by modifying the vascularization and altering the local immunity respectively[18,19]. Additionally, these niches play a role in keeping cancer cells dormant and triggering the development of tumours both locally or to distant organs by metastasis. This has been demonstrated in studies of OS that developed from benign lesions after patients underwent bone curettage and grafting, following long periods (7-28 years) of being disease free[20].

To explain the development of these secondary-induced primary bone tumours, the authors suggest that tumour growth was promoted by MSCs in the inserted scaffold. Perrot *et al*., also reported a delayed local reappearance of osteosarcoma. This came after 13 years from initial diagnosis, and 18 months following a lipofilling procedure. Perrot et al investigated the relationship between tumour growth, fat injections, and mesenchymal stem/stromal cell like cells present in fatty tissue. Results showed that fat grafts and progenitor cells promote tumour growth, indicating that de-regulation of tumour niches may reactivate tumour proliferation[21,22].An important element in the bone microenvironment is also the receptor activator of nuclear factor kappa-B ligand (RANKL) and its receptor RANK. RANKL is an essential mediator in osteoclast activation and differentiation, and consequently in bone remodelling. RANK is expressed by cells of monocyte lineage, endothelial cells, and also by OS tumour cells. A reverse correlation between the expression of RANK and overall patient survival suffering from OS but not with the response to chemotherapy has been demonstrated[23]. A recent study has however reported that RANK was not detectable in OS tumours. This absence suggests that any autocrine RANKL/RANK signalling in human OS tumour cells is not operative, and anti-RANKL therapy would not directly affect the tumour[24]. Pre-clinical investigations demonstrated that RANKL blockade by osteoprotegerin, or soluble RANK delivery has a strong impact on the tumour development[25,26]. In other cancer cell types, tumour-infiltrating regulatory T cells appear the main source of RANKL and may be a strong regulator of local immunity[27]. Therefore inhibition of RANKL can be an effective way to target the bone microenvironment. This has been demonstrated by using denosumab, a human monoclonal antibody specific to

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RANKL, which has been shown to prevent tumour-induced bone resorption and skeletal complications of metastatic bone disease arising from breast cancer[28].

Tumour Associated Macrophages

Cells of the immune system penetrate tumours to regulate the effects of inflammation and immunity[29]. The most abundant immune cells are macrophages, which were originally thought to generate anti-tumour activities by recruiting helper T-cells. However, clinical and experimental data have shown that certain macrophage phenotypes, are correlated with enhanced tumour progression, induction of angiogenesis and promotion of immunosuppression[30,31]

Macrophages demonstrate functional plasticity as a result of signals generated from stromal cells and tumour cells. They can differentiate into M1 or M2 type macrophages. M1 macrophages induce inflammatory responses and anti-tumor immunity, whilst M2 type induce anti-inflammatory responses and pro-tumorigenic properties through the induction of neo-angiogenesis. Macrophages that infiltrate tumours are known as tumour associated macrophages (TAMs). They resemble closely M2 type macrophages and are recruited to tumours as a result of overexpression of growth factors such as macrophage colony stimulating factor (M-CSF), CC Chemokine ligand 2 (CCL-2) and vascular endothelial growth factor (VEGF)[32]. The M2 subtype has been seen to promote the progression of cancer and minimize the efficacy of therapy using a combination of mechanisms. Primarily, they reinforce the presence of cancer cells by inhibiting anti-tumour responses and stimulate cell proliferation. Secondly, TAMs regulate angiogenesis by enhancing the angiogenic switch, and promoting the proliferation of endothelial cells. TAMs contribute to tumour progression by assisting in cancer cell invasion, seeding, extravasation, survival and proliferation of cancer cells at metastatic sites[31].

Tumour vascularization acts as an entry point for tumour cells to disseminate to other parts of the body and promote metastasis[29]. Additionally, TAMs accumulate in conditions of hypoxia within the tumour and up-regulate the expression of hypoxia-inducible factors, which in turn triggers transcription of various growth factors including VEGF[33]

In many cancers, the presence of TAMs leads to poor prognosis. However, it has also been recorded that the expression of TAM-associated genes in pre-treatment biopsies of OS, correlated with a lower risk of metastases. The authors observed an expression of macrophage-associated genes in hematopoietic cells and not in OS tumour cells. They also found that TAMs in post-chemotherapy resections and metastatic lesions, led to improved survival. The authors reported a heterogenous population of M1 and M2 phenotypes in OS tumours, and that there was an association between macrophage infiltration and higher micro-vessel density. This suggests that the influx of macrophages may support certain aspects of tumour growth in OS. However overall, in OS, direct or indirect antitumor activity of macrophages outweighs their possible tumour supporting effects[34]. Recently, Dumars et *al.* demonstrated the association of TAM to a better overall survival of OS patients[35]. These authors observed a dysregulation of the macrophage balance in favor of M1 cells in non-metastatic patients.

The above findings are backed by a clinical trial of 662 OS patients using muramyl tripeptide (MTP), a macrophage activating agent. Addition of this peptide to chemotherapy regimens of doxorubicin, cisplatin, and methotrexate resulted in a statistically significant improvement in 6-year overall survival, from 70% to 78% (P=0.3, hazard ratio = 0.71, 95% CI, 0.52

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to 0.96)[36]. It is therefore possible that the M1 and M2 macrophage ratio may regulate metastasis in OS, and that once a threshold of either phenotype is reached the tumour microenvironment may be changed to one that favors metastases. The results from this study are considered controversial, meaning that adjuvant MTP for OS has not been universally adopted and further investigation into this mechanism is needed.

Macrophage Colony-Stimulating Factor

The macrophage colony-stimulating factor (M-CSF or CSF-1) is a cytokine required for proliferation, differentiation and survival of cells from the hematopoietic lineage including monocytes, macrophages, and osteoclasts[37]. The effects of M-CSF are regulated through a type III tyrosine kinase receptor called MCSF-R, (also known as c-fms, CD115 and CSF-1R) which is encoded by the proto-oncogene *c-fms*. The importance of M-CSF has been demonstrated *in vivo* using mutant osteopetrotic *(op/op)* mice[38]. The mice exhibited a number of skeletal abnormalities (e.g. stunted growth, domed skull, stubby appearance of the tarsals, metatarsals, femur and humerus), a toothless phenotype, and deficiencies in macrophages and osteoclasts. This phenotype resulted from a null mutation in the *CSF-1* gene by insertion of a single base pair, and led to a deficiency in the production of osteoclasts[37]. Preliminary experiments indicated that the above effects might be restored by injecting the recombinant form of human M-CSF to the *op/op* mice. This resulted in correction of the observed osteopetrotic phenotype, as well as restoration of the number of macrophages and osteoclasts[38]. However it did not overcome all the defects observed in the *op/op* mice, indicating that other variants of M-CSF or other cytokines, might be acting in combination to regulate the activity of osteoclast.

M-CSF in Cancer

Lymphocytes, osteoblasts and stromal cells secrete M-CSF in order to sustain the continuous proliferation of the tumour by a direct or indirect effect depending on the expression of M-CSFR in cancer cells. M-CSF can act as an autocrine, paracrine and endocrine factor. Increased expression of M-CSF has been found in a number of cancers including breast, pancreatic and colorectal cancer. High expression levels of M-CSF in ovarian cancer correlate with increased tumour aggressiveness and poorer prognosis[39].

M-CSF has also been suspected in the process of tumour metastasis in breast cancer. A recessive null mutation of CSF-1 gene resulted in delayed lung metastasis and tumour progression in a murine breast cancer model. This reduction was explained by the authors as arising due to lack of TAMs (M1 type). Restoring local concentrations of MCS-F, resulted in the promotion of tumour development. These pro-tumoral actions are exerted through macrophages, suggesting that lack of macrophages in tumours of Csf1op/Csf1op mice is primarily due to the systematic loss of CSF-1, and that other chemo attractants are present in mice to recruit macrophages into the tumour site[40].

Other studies have reported that M-CSF has the potential to bring about anti-tumour responses as well. Rat T9 glioma cells transfected with membrane bound isoforms of macrophage M-CSF (mMCSF; a non-secreted isoform of M-CSF) were killed by macrophages in a dose dependent manner. Killing of mM-CSF expressing tumour cells by macrophage in vitro occurred through phagocytosis[41]. Although these reports are contradictory, the tumour promoting actions of M-CSF are well documented, and overall it is regarded as a “pro-tumor” cytokine. Another cytokine which promotes the differentiation, proliferation, and functional regulation of monocytes, macrophages

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and dendritic cells, is the recently discovered IL-34.

Interleukin-34

In 2008, a novel cytokine, Interleukin-34 (IL-34) was discovered by producing recombinant forms of proteins from cDNA's encoding both secreted proteins and extracellular domains of trans-membrane proteins. They transfected these into HEK 293T cells and screened their biological activities through a number of cell-based assays. They demonstrated that IL-34 transduced signaling pathways through MCSF-R receptor. They also showed that IL-34 induced the formation of colony forming unit macrophages in human bone marrow cultures with the same effectiveness as M-CSF[42]. In light of this study, it was hypothesized that IL-34 shared common features with M-CSF and revealed a functional overlap.

Functional studies demonstrated that both M-CSF and IL-34 stimulate macrophage differentiation and up-regulate monocyte activity[42,43]. However several phenotypic differences were observed in the resulting macrophages. These differences predicted that IL-34 uses an alternative binding mode from M-CSF on binding to the MCSF-R receptor. Structural analysis showed that IL-34 and M-CSF bind to the extracellular domain of MCSF-R in a similar way, but through two distinct contact points. Binding of IL-34 or M-CSF to MCSF-R leads to receptor dimerization and differential auto-phosphorylation on its eight tyrosine residues[44] (Figure 1A). M-CSF/MCSF-R and IL-34/MCSF-R crystals have a similar shape, but the IL-34/M-CSFR complex is more stable. Chihara et al. showed some differences in the kinetics of MCSF-R phosphorylations and in the nature and intensity of phosphorylated tyrosine residues after IL-34 binding, partly explaining the differences in the signaling pathways they elicit, and also revealing in part their functional overlap.

An interesting observation comes from the study of Wei et al., in 2010, which provides new insights on the expression patterns of M-CSF and IL-34. When studying the expression levels of these two cytokines during mouse development, they showed that their expression levels differ substantially in a spatiotemporal manner. Thus IL-34 although it has overlapping functions with M-CSF, could be acting to coordinate the cellular communication network between osteoblasts, macrophages and osteoclasts at the microenvironment level rather than at the systemic level. This however, needs to be elucidated further since it might have further implications on the bone niche and how IL-34 plays a role in maintaining the survival and proliferation of sarcoma cells.

IL-34 is highly expressed in post-natal and adult brains and concurrently, MCSF-R is also highly present in early development, but dramatically decreases, almost undetected, in adult brains. This high expression of IL-34 in adult brains without expression of its receptor, suggested that other receptors for this cytokine exists[45]. The receptor protein tyrosine phosphatase (RPTPβ/ζ) was recently identified on the glioblastoma cell line U251, as another receptor for IL-34 through its cell surface chondroitin sulfate (CS) chains. By using IL-34 affinity chromatography of solubilized mouse brain membrane followed by mass spectrometric analysis, Nandi et al reported that IL-34 selectively binds to cell surface PTP-ζ and initiates downstream signalling leading to inhibition of cell proliferation and motility. They also showed that IL-34 binding to PTP-ζ is dependent on the presence of CS chains. Similarly, syndecan-1, also a proteoglycan with CS chains, was able to modulate IL-34-induced M-CSFR signalling pathways. Syndecan-1 also increases the migration M2 macrophages induced by IL-34. In addition, it was proved that IL-34 induced myeloid cell migration, is dependent on syndecan-1[46]. Therefore in addition to MCSF-R, RPTPβ/ζ and syndecan-1 are

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key regulators of IL-34 activity, and may play a role in bone inflammatory diseases and bone sarcoma development (Fig 1B and 1C).

Role of IL-34 And M-CSF In Bone Biology

In addition to the major role played by these cytokines in macrophage proliferation and differentiation, M-CSF and IL-34 play a central part in bone remodeling through osteoclastogenesis, the process by which osteoclasts break down bone. In association with RANKL, IL-34 can replace M-CSF to induce osteoclast formation by stimulating the proliferation and adhesion of osteoclast precursors. IL-34 can completely substitute for M-CSF during this process, thus defining a novel pathway for osteoclastogenesis. IL-34 was highly expressed in osteoclast-like cells found in giant cell tumours of bone. In contrast to osteoblasts, osteoclasts showed very strong staining for IL-34, suggesting a potential role in the pathogenesis of bone sarcomas by promoting osteoclast formation[47]. Osteoclastogenesis and the differentiation of osteoclasts is mainly dependent on M-CSF and RANKL, a TNF family cytokine. The role of M-CSF in osteoclastogenesis, as demonstrated in the osteopetrotic op/op mice, was previously described[38]. Similarly IL-34 can contribute to osteoclastogenesis. It was demonstrated that IL-34 expression was able to recover the main defects observed in op/op mice[42]. Using *in vitro* murine and human models of osteotogenesis[47], IL-34 was able to support RANKL-induced osteoclastogenesis in the absence of M-CSF. IL-34 stimulated RANKL-induced osteoclastogenesis by promoting the adhesion and proliferation of osteoclasts progenitors, solidifying further the hypothesis that M-CSF and IL-34 exhibit a functional overlap.

Similar to M-CSF, IL-34 was shown to promote the differentiation of monocytes into immunosuppressive M2 macrophages[48] and to up-regulate IL-6 and chemokines P-10/CXCL10, IL-8/CXCL8 and MCP-1/CCL2 in human whole blood[49]. Recently, it was also demonstrated that both cytokines induced similar effects on macrophage differentiation ability but was associated with a different polarization potential

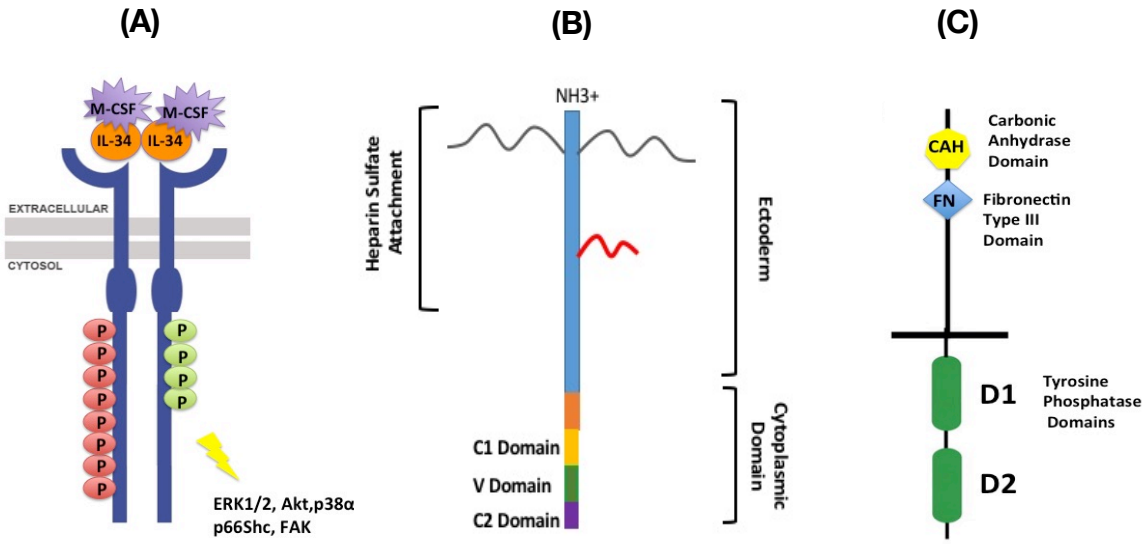

Figure1: The receptors of Interleukin-34. Diagram showing the three proposed receptors for IL-34**. (A)** MCSF-R Receptor; IL-34 and M-CSF bind to the MCSF-R receptor by two distinct

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contact points. Binding induces the auto-phosphorylation of different tyrosine residues (red for M-CSF and green for IL-34) and subsequently differential biological responses **(B)** Syndecan-1; a proteoglycan with CS chains, able to regulate IL-34-induced M-CSFR signalling pathways. Syndecan-1 also increases the migration of M2 macrophages induced by IL-34. **(C)** RPTPβ/ζ; Binding of IL-34 to PTP-ζ is dependent on the presence of CS chains. IL-34 selectively binds to cell surface PTP-ζ and initiates downstream signalling leading to inhibition of cell proliferation and motility. MCSF-R, RPTPβ/ζ and syndecan-1 are key regulators of IL-34 activity, and may play a role in bone inflammatory diseases and bone sarcoma development.

This suggests both IL-34 and M-CSF are key regulators of inflammation. IL-34 overlaps with the roles played by M-CSF in inflammation in degenerative bone diseases such as rheumatoid arthritis and periodontal inflammation. The role of IL-34 in rheumatoid arthritis was confirmed when cytokine levels where significantly higher in the synovial fluids of rheumatoid arthritis patients in comparison to osteoarthritis patients, and correlated with leucocyte number and inflammation intensity[51,52]. In periodontal inflammation, IL-34 was found to be expressed in gingival fibroblasts, and its expression enhanced by the pro-inflammatory cytokines TNF-α and IL-1β They also confirmed once more that IL-34 was able to support RANKL-induced osteoclastogenesis of bone marrow macrophages independently of M-CSF[53].

The Role Of IL-34 In Osteosarcoma

IL-34 is expressed in a number of cancers such as breast, ovarian, colorectal, lung, skin, and brain[54] and plays an important role in the tumour microenvironment through alternations of the bone, immune and vascular niches. One of the main mechanisms by which IL-34 acts on cancer cells, is by recruiting M2-polarised macrophages and promoting formation of new blood vessels and extravasation of immune cells. This has been demonstrated in osteosarcoma where in vitro effects were studied using endothelial cell precursors (ECFC) and human umbilical vascular endothelial cells (HUVEC) to determine the role IL-34 in angiogenesis and the adhesion of monocyte to endothelium respectively. IL-34 stimulated the proliferation of endothelial cells and vascular cord formation in cultures of ECFCs, and increased the number of adherent monocytes in co-cultures with HUVECs. More importantly were the results from *in vivo* studies using mouse models inoculated with OS cells overexpressing IL-34. In comparison to control OS cells, OS cells overexpressing IL-34 resulted in larger primary tumours and increased number of lung metastases. *In vivo,* IL-34 increased the recruitment of M2 TAMs into the tumour tissue[55].

IL-34 also functions in an autocrine manner by acting on CSFR-1 expressing tumour cells and TAMs. IL-34 induces the phosphorylation of CSFR-1 which in turn leads to activation of C/EBPβ via the AKT mediated pathway. This enhances the pro-tumourigenic and immunosuppressive functions of TAMs which consequently contributed to therapeutic resistance in cancers. This helps overcome strict conditions of chemotherapy such as that observed in lung cancers. IL-34 was detected at high levels in cells rendered resistant to chemotherapeutic agents[56,57]. In addition, expression of IL-34 was correlated with tumour progression and poor survival in lung cancer patients[57]. Recently, Franze et al. showed that IL-34 was able to support pro-tumourigenic signal in colon cancer[58]. IL-34 expression in tumours may therefore be a critical prognostic biomarker correlating with tumour malignancy. In light of the pro-tumourigenic functions described above, Il-34 makes an attractive therapeutic target. Inhibition of CSF-1 targeting alone,

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will be insufficient to block the signaling of IL-34 since IL-34 binds to several other receptors including syndecan-1 and *RPTPβ/ζ* which are frequently expressed in various cancers. Thus targeting the signaling pathway that controls the production of IL-34 in the tumour microenvironment may be effective in multi-agent chemotherapy.

Table 1: A comparison of the characteristics of Interleukin-34 (IL-34) and macrophage colony stimulating factor (M-CSF)[59]

	IL-34	CSF-1
Location	Chromosome 16	Chromosome 1
Size	242 aa, 27.5 KDa	554 aa, 60 KDa
Isoforms	2 isoforms	3 isoforms
Subunit structure	N-glycosylated homodimer	Homodimer or heterodimer, disulfide linked
Gene Product	Extracellular space	Extracellular space, extracellular exosome, cell membrane, perinuclear region of cytoplasm, plasma membrane
mRNA expression	Predominantly expressed in spleen*	
Receptors	M-CSFR Syndecan-1 RPTPβ/ζ	MCSF-R

* for IL-34 and M-CSF. Both cytokines are also detected in a range of other tissues including heart, brain, lung, liver, kidney, thymus, testis, ovary, small intestine, prostate and colon.

CONCLUSION AND FUTURE DIRECTIONS

Over the past few years, increasing evidence has solidified the role of IL-34 as a cytokine playing important roles in bone biology and in the pathophysiology of diseases. IL-34 increases monocyte/macrophage survival and

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viability, and can substitute for MCS-F in RANK-L induced osteoclastogenesis. Primarily, IL-34 uses the MSCF-R receptor to induce the activation of downstream signaling pathways. Additionally, two other receptors for IL-34 are present: syndecan-1 and RPTPβ/ζ. These receptors however, are not specific to the cytokine, and thus a receptor specific for IL-34 is still expected to be recognized. IL-34 has been shown to play important roles in the pathogenicity of diseases associated with inflammation, whilst in cancers, Il-34 is also found to be a key regulator. In osteosarcoma, Il-34 promotes tumour progression and metastasis by macrophage recruitment, promoting angiogenesis and chemotherapeutic resistance. Studies are also increasingly suggesting that IL-34 might be specifically coordinating the communication network at the tumour microenvironment. The future of IL-34 lies in exploring new therapeutic strategies to inhibit signaling pathways and/or controlling its production. By selectively blocking IL-34 or MCS-F, this approach may provide more flexibility than blocking MCSF-R alone. Therapeutic combination between anti IL-34 and conventional chemotherapy can then be envisaged. Thus, blocking IL-34 is a highly promising area for improving drug therapies and personalized treatments for osteosarcoma patients, especially for those with metastatic disease.

CONFLICT OF INTEREST

None Reported.

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ABBREVIATIONS

OS, Osteosarcoma; TP53, Tumour Protein P53; RB1, Retinoblastoma; RECQL4, REC Q Helicase 4; CDK4, Cyclin-Dependent Kinase 4; MDM2, Murine Double-Minute Type 2; MSC, Mesenchymal Stem Cells; RANKL, Receptor Activator of Nuclear Kappa-B Ligand; mAb, Monoclonal Antibody; TAM, Tumour Associated Macrophages; M-CSF, Macrophage- Colony Stimulating Factor; CSF-1, Colony Stimulating Factor-1; CCL-2, Chemokine ligand 2; VEGF, Vascular Endothelial Growth Factor; MTP, Murayml Tripeptide; MCSF-R, Macrophage-Colony Stimulating Factor Receptor; FGF, Fibroblast Growth Factor; TGF, Transforming Growth factor; mMCS-F, Membrane Macrophage-Colony Stimulating Factor; IL-34, Interleukin-34; cDNA, Complementary DNA; TNFα, Tumour Necrosis Factor Alpha; IL-1β, Interleukin – 1 Beta; ECFC, Endothelial Colony Forming Cells; HUVEC, Human Umbilical Vein Endothelial Cells; RPTPβ/ζ, Receptor Protein Tyrosine Phosphatase Beta/Zeta; CS, Chondroitin Sulfate.

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