



# Protein Cyt11: its role in chondrogenesis, cartilage homeostasis, and disease

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## Abstract

Cytokine-like protein 1 (Cyt11), also named Protein C17 or C4orf4 is located on human chromosome 4p15-p16 and encodes a polypeptide of 126 amino acid residues that displays characteristics of a secretory protein. Cyt11 is expressed by a sub-population of CD34<sup>+</sup> human mononuclear cells from bone marrow and cord blood, and by chondrocytes (cartilage-forming cells). In this review, we explore evidence suggesting that Cyt11 may be involved in the regulation of chondrogenesis, cartilage homeostasis and osteoarthritis progression, accompanied by the modulation of Sox9 and insulin-like growth factor 1 expression. In addition, Cyt11 exhibits chemotactic and pro-angiogenic biological effects. Interestingly, CCR2 (C–C chemokine receptor type 2) has been identified as a likely receptor for Cyt11, which mediates the ERK signalling pathway. Cyt11 also appears to mediate the TGF-beta-Smad signalling pathway, which is hypothetically independent of the CCR2 receptor. More recently, studies have also potentially linked Cyt11 with a variety of conditions including cardiac fibrosis, smoking, alcohol dependence risk, and tumours such as benign prostatic hypertrophy, lung squamous cell carcinoma, neuroblastoma and familial colorectal cancer. Defining the molecular structure of Cyt11 and its role in disease pathogenesis will help us to design therapeutic approaches for Cyt11-associated pathological conditions.

**Keywords** Birth · Heart · Immune · Toxic · Genetic

## Introduction

Cytokine-like protein 1 (Cyt11) was initially discovered in relation to a rare population of human bone marrow (BM) and cord blood (CB) mononuclear cells that function as haematopoietic stem/progenitor cells and bear the CD34 surface marker (CD34<sup>+</sup>) [1]. The Cyt11 gene, also called the C17 gene, is not expressed in mature haematopoietic cells that lack CD34 expression, and was identified to elucidate

possible molecular mechanisms regulating the maturation of haematopoietic progenitor cells [1]. Although the Cyt11 gene has been mapped to human chromosome 4p15–p16, the definitive molecular structure of the Cyt11 protein remains to be determined, and there is uncertainty as to the true classification of Cyt11 due to its cytokine-like properties and chemokine activity.

Evidence indicates that Cyt11 is a secretory protein with a predicted structure characteristic of haematopoietic cytokines and interleukins, and capable of performing immunobiological functions [1]. The cellular signalling mechanisms and potential receptors for Cyt11 remain largely unknown. Cyt11 appears to play a role in chondrogenesis, cartilage homeostasis, and osteoarthritis progression. Recent research has found that the Cyt11 gene may increase individual susceptibility to a number of systemic diseases, and function in an immunomodulatory capacity with implications for toxicology relating to early childhood illness. Further evidence suggests that Cyt11 may have an emerging role in tissue regeneration and as a target for the delivery of therapeutic agents for disease control.

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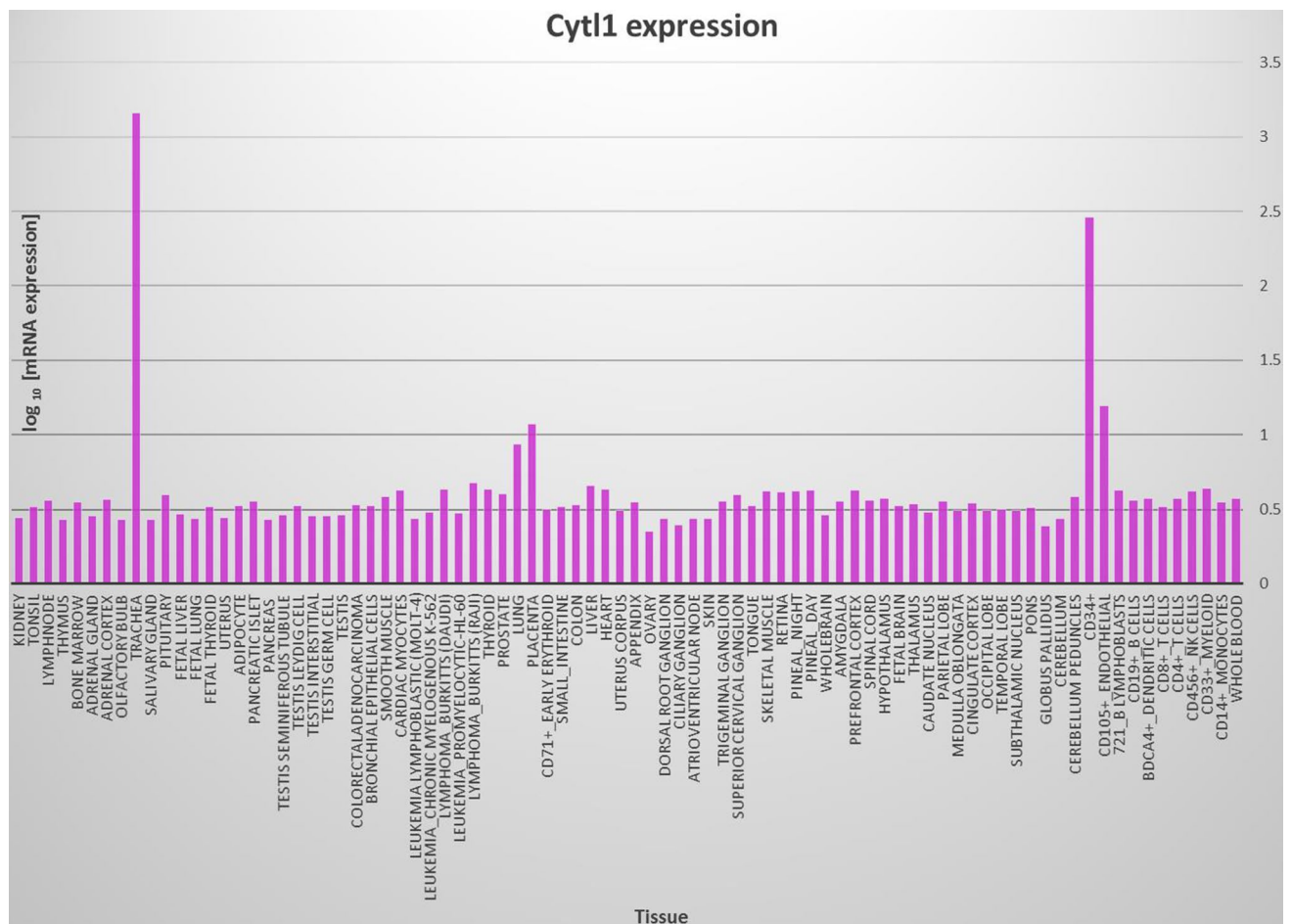
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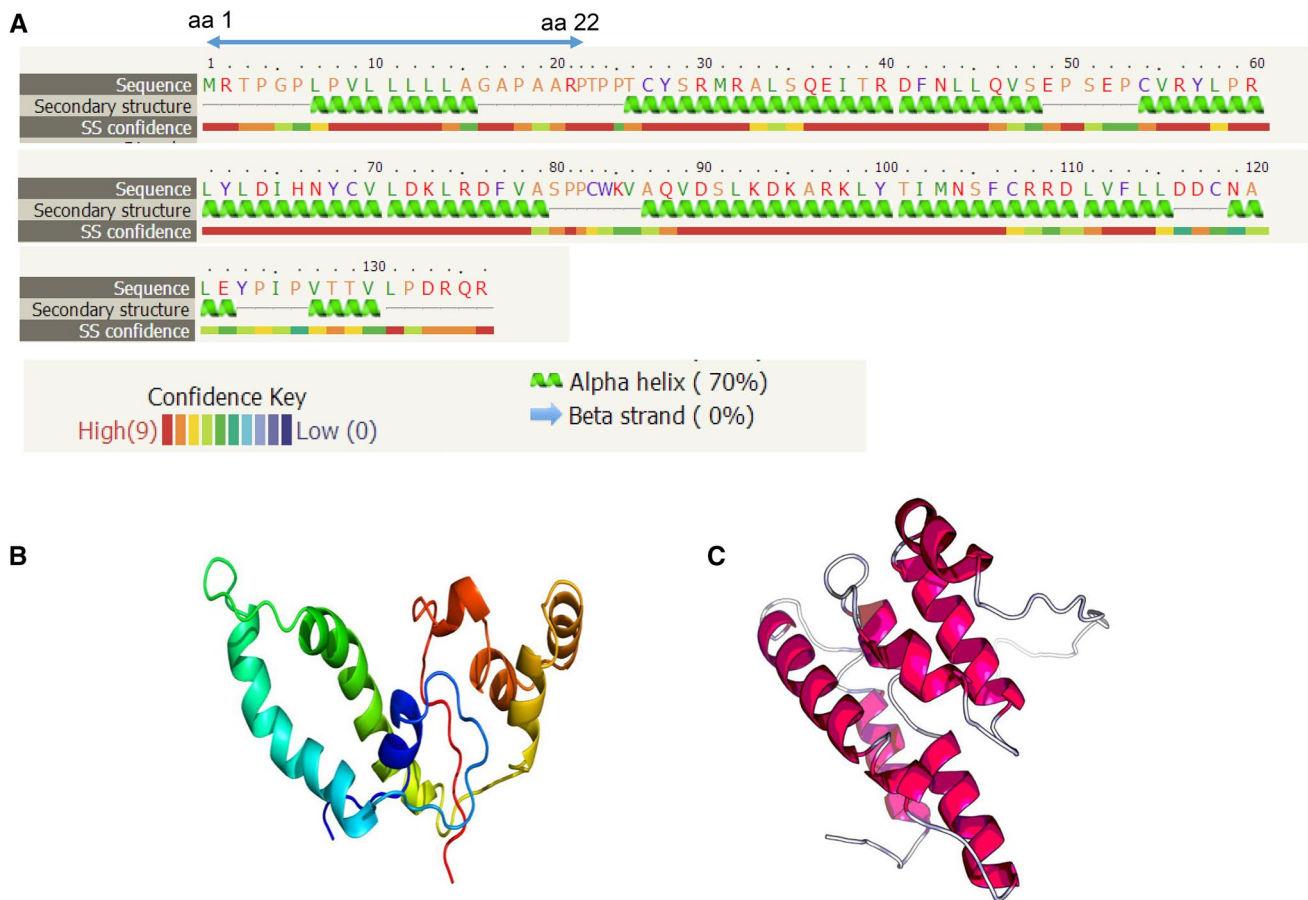
**Fig. 2** BioGPS analyses showing the expression profiling of human gene among various tissues and cells. Note that Cyt11 is most specifically expressed in trachea, a cartilage-rich tissue, and in CD34+ cells

transcriptional activity and the expression of insulin-like growth factor 1 [7] (Fig. 6).

Structurally, Cyt11 appears to resemble an IL8-like chemokine folding, with particular similarity to monocyte chemoattractant protein 1, MCP-1 (or CCL2), and appears to have a functional chemokine receptor, CCR2, that is likely to be involved in the pathogenesis of osteoarthritis and rheumatoid arthritis [8]. Recent research has shown that Cyt11 exhibits a chemotactic effect on monocytes and macrophages via its receptor CCR2B-ERK signalling pathway [9] (Fig. 6). This direct signalling effect is further confirmed because macrophages from wild-type mice but not from CCR2 null mice are chemotactically responsive to recombinant Cyt11 proteins [9].

Interestingly, Cyt11, also appears to be involved in the pathogenesis of cardiac fibrosis (CF) and heart failure (HF), and may be structurally and functionally related to MCP-1 (or CCL2) [10]. Cyt11 has been shown to induce the expression of TGF- $\beta$ 2, and appears to play an

essential role in CF and HF via activation of the TGF- $\beta$ -Smad signalling pathway [10]. Further, the pro-fibrotic activity of Cyt11 was unaffected by antagonization of the CCR2 receptor, which indicates that Cyt11 may have more than two receptors, and that the receptor that is responsible for mediating the pro-fibrotic activity of Cyt11 in the heart remains to be identified [10] (Fig. 6). In addition, Cyt11 appears to be produced by endothelial progenitor cells, particularly endothelial colony-forming cells (ECFCs), and was found to have pro-angiogenic effects by inducing sprouting and vessel formation that is comparable to VEGF-A [11]. O-glycosylation on two neighbouring threonines in the C-terminus appears to be important for the pro-angiogenic bioactivity of Cyt11 [11]. It is suggested that this angiogenic function is largely independent of VEGF-A [11]. Thus further studies are required to demonstrate the detailed mechanisms by which Cyt11 may mediate pro-angiogenic functions and may be implicated in the pathogenesis of cardiac fibrosis and heart failure.



**Fig. 3** Molecular structure of Cyt11. **a** Secondary structure of Cyt11 showing that it contains a signalling sequence from amino acid residues aa 1 to aa 22, and mainly three large alpha helix domains and two small motifs with no predicted beta strand according to the web-link-based bioinformatics analysis (<http://twitter.com/phyre2server>). **b** Tertiary structure predicted by RaptorX template-based protein struc-

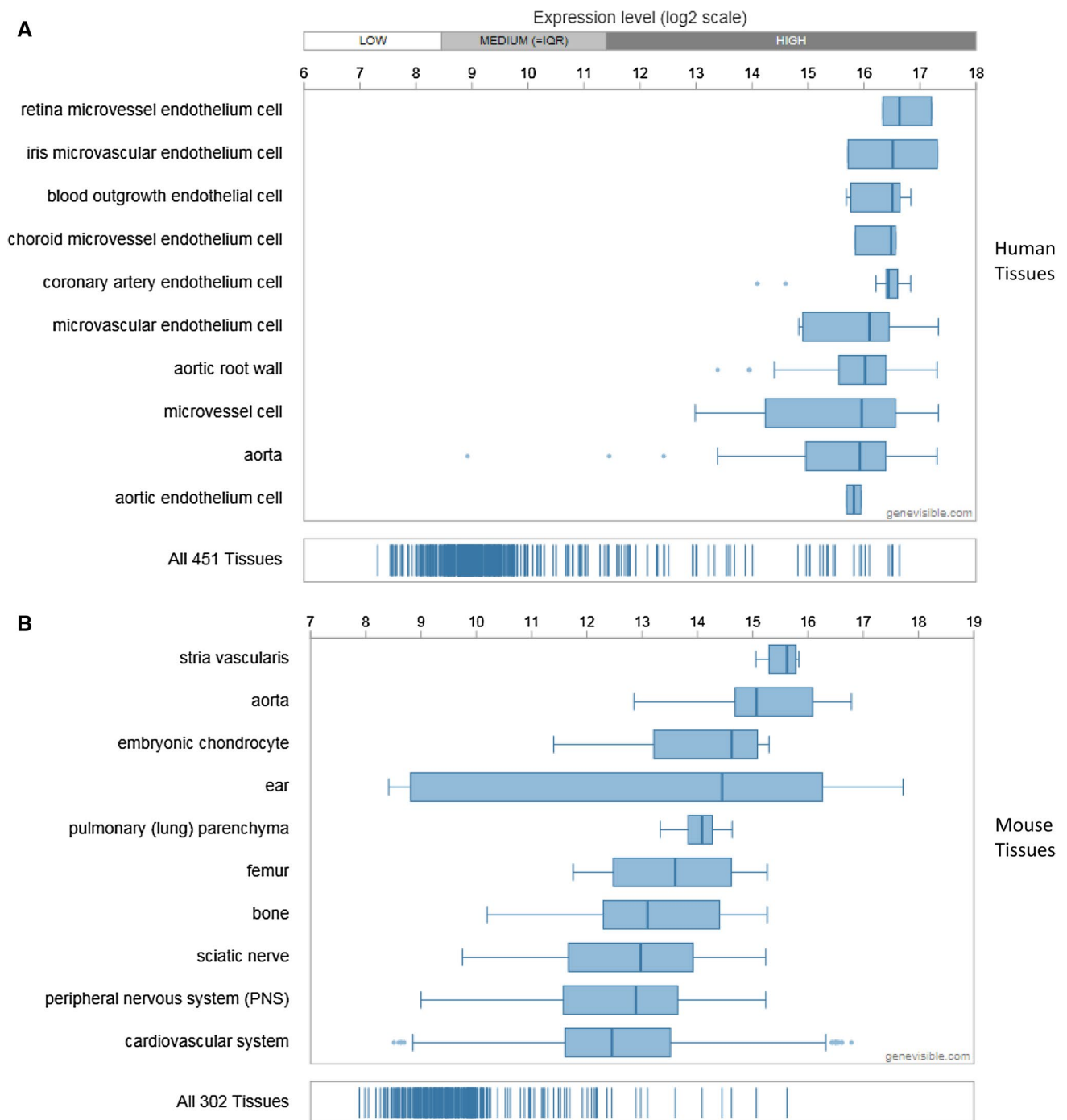
ture modelling according to the web-link based bioinformatics analysis (<http://raptorx.uchicago.edu/StructurePrediction>). **c** Tertiary structure predicted by the Phyre2 web portal for protein modelling based on the web-link bioinformatics analysis (<http://twitter.com/phyre2server>)

## The role of Cyt11 in cartilage homeostasis and osteoarthritis development

Osteoarthritis (OA) is a chronic debilitating joint disease characterized by the degradation of articular cartilage, subchondral bone sclerosis, osteophyte formation, and inflammation of the synovial membrane [12]. Further, research implicates invasive angiogenesis, the critical role of pro-inflammatory secretory molecules, and chondrocyte dedifferentiation in the pathophysiology of OA [13–15]. Cyt11 is known to regulate the chondrogenic differentiation of mesenchymal cells and increase the expression of interleukin-1 (IL-1) during chondrogenesis [7]. Additionally, Cyt11 appears to have strong pro-angiogenic effects, comparable to vascular endothelial growth factor-A (VEGF-A), has chemotactic activity towards monocytes and macrophages via the CCR2B receptor, and its expression appears to be downregulated during OA progression [9, 11, 16]. Taken

together, these findings suggest that Cyt11 is implicated in the progression of OA and could be a potential therapeutic target for intervention.

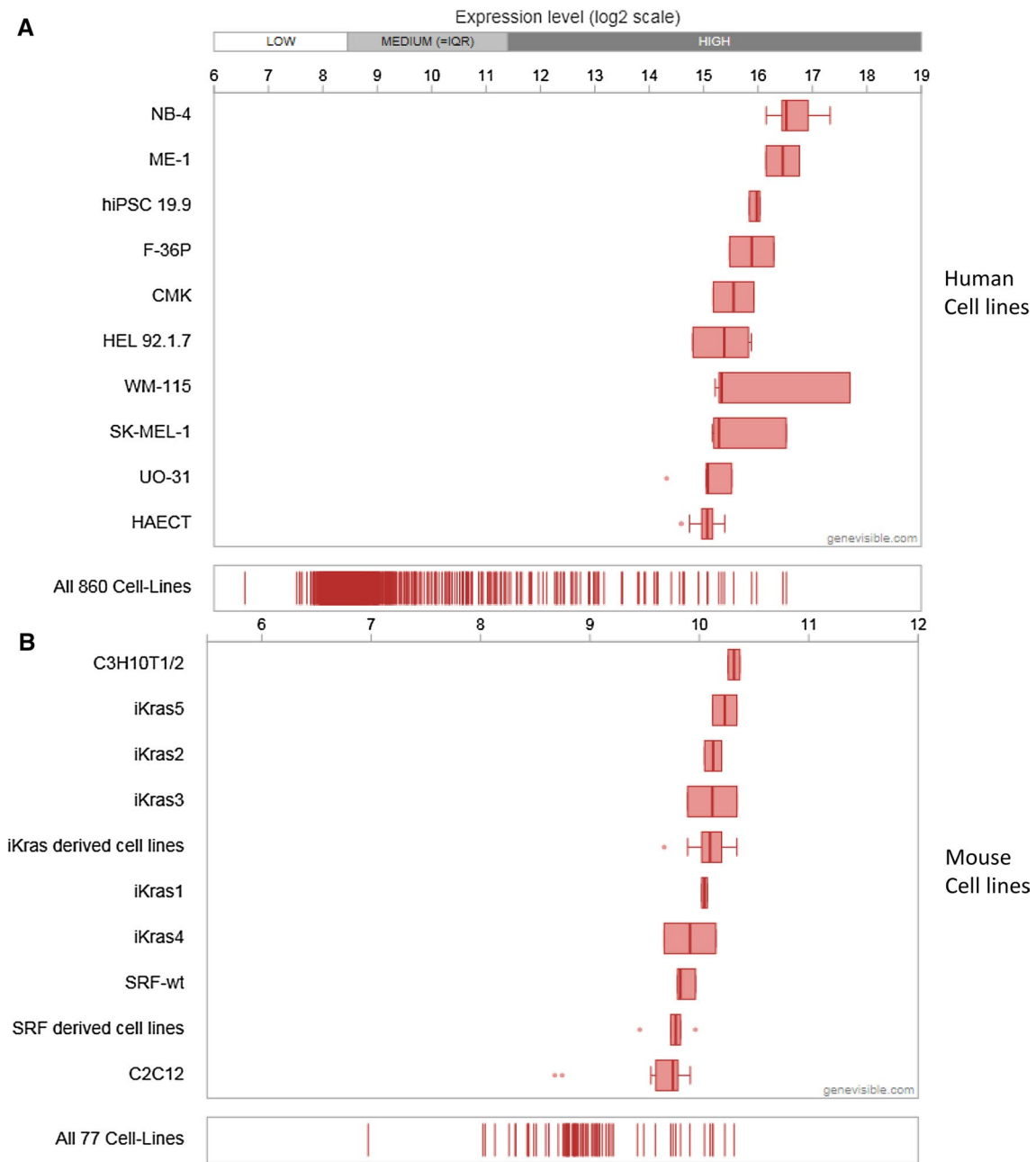
Although Cyt11 was initially found to be expressed by a human population of CD34<sup>+</sup> bone marrow and cord blood cells, Cyt11 was more recently reported to be expressed by chondrocytes, which is suggestive of a role in chondrogenesis and cartilage homeostasis [1, 17, 18]. However, Cyt11 gene knockout did not affect chondrogenesis or cartilage development as Cyt11 (–/–) mice exhibited normal endochondral ossification and long bone development with no abnormal ultrastructural features of articular cartilage, including matrix organization and chondrocyte morphology [6]. Notably, Cyt11 (–/–) mice showed more cartilage destruction after the medial meniscus of mouse knee joints was disturbed, as compared with wild-type littermates [6]. Further, Cyt11 was found to be preferentially expressed in chondrocytes and in cartilage-rich tissues,



**Fig. 4** Expression analyses performed by Genevisible (<http://genevisible.com>) showing the Cyt11 expression in both human and mouse tissues, including the 10 most highly expressed tissues for each species

and systemic expression of Cyt11 *in vivo* inhibits collagen antibody-induced arthritis in mice [18]. More recently, Cyt11, was identified as being differentially expressed in post-traumatic osteoarthritis (PTOA), indicative that Cyt11 may be a potential target for specific intervention in early PTOA, and further suggesting the role of Cyt11 in osteoarthritis development [16]. Also, the expression

levels of Cyt11 were decreased in OA cartilage of humans and experimental mice [6]. Collectively, multiple lines of evidence suggest that, rather than regulating physiological cartilage and bone development, Cyt11 is involved mainly in the maintenance of cartilage homeostasis, and that loss of Cyt11 function is associated with osteoarthritis progression [6, 7, 18, 19].

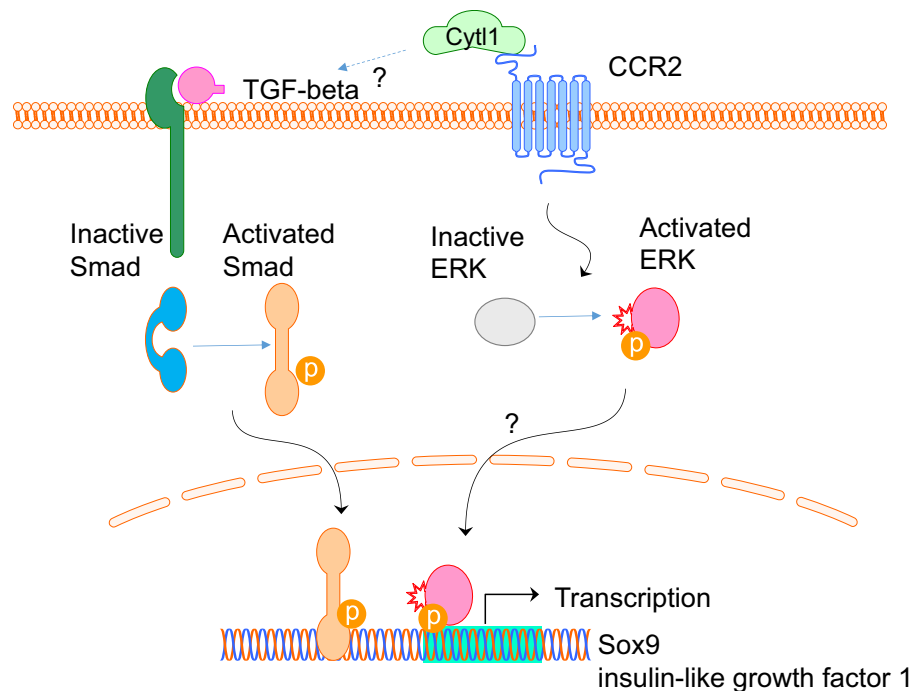


**Fig. 5** Expression analyses performed by Genevisible® <http://genevisible.com> showing the Cyt11 expression in both human and mouse cell lines, including the 10 most highly expressed cell lines for each species

In addition to its role in cartilage homeostasis and osteoarthritis progression, Cyt11 has been found to be involved in the regulation of macrophage migration [8, 9, 20]. Structural–functional analyses of Cyt11 suggests that it is likely to adopt an IL8-like chemokine fold, in particular similar to CCL2 (monocyte chemoattractant protein 1, MCP-1), and Cyt11 consistently appears to exhibit a chemotactic effect for monocytes and macrophages [8, 9]. Thus, Cyt11 appears to have cytokine-like properties combined with chemokine abilities, and, therefore, definitive determination of the tertiary

structure of Cyt11 is required to make a true classification [20]. Current evidence indicates that Cyt11 may be an important atypical cytokine-like protein ligand with functional chemokine receptor, CCR2 [20].

**Fig. 6** Putative signalling pathway of Cyt11. Cyt11 appears to act on the CCR2 receptor to activate ERK signalling and downstream transcriptional activation of Sox9 and insulin-like growth factor 1. Alternatively, Cyt11 might also act via the TGF-beta-Smad signalling pathway, independent of CCR2, to activate downstream transcriptional activation of Sox9 and insulin-like growth factor 1



## The potential role of Cyt11 in cancers and disease pathogenesis

Cyt11 is associated with several types of cancers [21–24]. It was identified as one of the three candidate genes that harboured rare loss-of-function variants in both early-onset and familial colorectal cancer (CRC) in a study analysing in 3374 Finnish and 58,112 non-Finnish controls [21]. Using a combination of the whole-genome DNA methylation pattern and the gene-expression profile, Cyt11 was also identified as one of the candidate genes that is regulated by DNA methylation in human lung squamous cell carcinoma [22]. In addition, Cyt11 gene expression was detected in human tumour cell lines, particularly SH-SY5Y, and human neuroblastoma (NB) tissues, and decreased expression of the Cyt11 gene when blocked by siRNA results in reduced cell proliferation, migration and invasion activities by SH-SY5Y cells [24]. These findings are indicative of an association of Cyt11 expression with growth and metastasis of neuroblastoma cells, and suggest that Cyt11 may be a potential therapeutic target and diagnosis biomarker for NB [24]. Further, previous studies have revealed that Cyt11 expression together with secreted CXC-type chemokines was associated with benign prostatic hypertrophy [23].

A pro-angiogenic function of Cyt11 was identified in endothelial progenitors, suggestive of its role in vessel formation and thus its applicable potential for tissue regeneration in ischemic pathologies [11]. In addition, Cyt11 was also found expressed in the endometrium as an ovarian hormone-dependent gene, and regulates the proliferation of HEC-1-A

and RL95-2 cells, suggesting a putative role of Cyt11 in the regulation of embryo implantation during early pregnancy [17]. Further, differential expression of Cyt11, together with Efnb1, and Tex26, was found in trophoblast biopsies, which suggests that Cyt11 might be associated with successful embryo implantation and live birth [25].

Alcohol dependence (AD) is a complex disease, with genetic and environmental aetiological factors, resulting in uncontrolled alcohol consumption. A recent genome-wide association study (GWAS) found that certain haplotypes within the Cyt11 gene were significantly associated with an increased risk of alcohol dependence (AD), suggesting that the Cyt11 gene may play an important role in the susceptibility to AD [26]. The possible underlying molecular and cellular mechanisms of this association are unknown and further studies are required to elucidate the significance of Cyt11 gene variants in relation to AD.

Tobacco smoking is a risk factor for diseases including cancer, cardiovascular disease, and diabetes. A recent twin study investigating DNA methylation and gene expression markers in tissues outside the lung, found that Cyt11 is one of five genes, including Ahrr, Cyp1a1, Cyp1b1, and F2r13, that appear to be hypomethylated and upregulated in smokers, indicating that DNA methylation and expression levels of Cyt11 may serve as an associated marker of smoking-associated impaired metabolic health [27].

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are synthetic fluorinated compounds that have been produced in large-scale quantities for consumer-products and industrial use, and are considered environmental pollutants with toxic

properties [28]. Prenatal human exposure to PFAS occurs via placental transfer and is associated with postnatal and early childhood immunosuppression [28]. Cyt11 is one of several immunomodulatory genes that were also found to be PFAS exposure-associated genes that are associated with early childhood immunosuppression and increased susceptibility to common colds [28]. Therefore, Cyt11 appears to be one of several immunomodulatory genes that might serve as a PFAS toxicogenomics marker [28].

Consistent with the viewpoint that Cyt11 has immune-regulatory properties: Cyt11 bears sequence homology to IL-2, may be a member of the IL-2 cytokine family, and appears to contribute to immune homeostasis systemically, and in a tissue-specific capacity [18]. Further investigation of the immunobiological functions, potential receptors, and signalling pathways of Cyt11 is required for this relatively novel protein [9]. Understanding the mechanisms by which Cyt11 regulates these pathological conditions will be important in designing therapeutic targets for Cyt11.

## Conclusion

Cytokine-like 1 is a secretory protein that was discovered by its expression in a sub-population of CD34<sup>+</sup> haematopoietic progenitor/stem cells, and appears to be expressed mainly in CD34<sup>+</sup> cells, chondrocytes, and cartilage-rich tissues [1, 7]. Cyt11 is located on human chromosome 4p15–p16, and has cytokine properties and chemokine activity, supporting the hypothesis that Cyt11 functions via immune-regulation. Bioinformatics analyses indicate that Cyt11 is expressed in human and mouse tissues, notably ocular microvascular endothelium, cardiovascular, and cartilaginous tissue. Cyt11 appears to regulate chondrogenesis via stimulating Sox9 and insulin-like growth factor 1 transcriptional activity [7]. Collective evidence suggests that Cyt11 may induce chondrogenesis, and is required for cartilage homeostasis, and the prevention of osteoarthritis progression, rather than the regulation of cartilage and bone development [6]. Cyt11 appears to have a functional chemokine receptor, CCR2, which may regulate ERK signalling to stimulate Sox9 and insulin-like growth factor 1 transcriptional activity. In addition, Cyt11 also appears to activate the TGF-beta-SMAD signalling pathway via an unidentified receptor, and play an important role in cardiac fibrosis and heart failure. Recent research indicates that Cyt11 may be associated with a number of cancers and disease pathogenesises, including neuroblastoma, alcohol dependence, and smoking-associated impaired metabolic health. Cyt11 may also be associated with successful embryo implantation and live birth. Further research is necessary to explore Cyt11 as a potential target for the delivery of therapeutic agents for tissue regeneration and disease control.

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## Compliance with ethical standards

**Conflict of interest** All authors declare that they have no conflict of interest.

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