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# The identification of articular cartilage and growth plate extracellular matrix-specific proteins supportive of either osteogenesis or stable chondrogenesis of stem cells

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ABSTRACT

Tissue-specific extracellular matrix (ECM) proteins can play a key role in regulating the fate of stem cells and can potentially be utilized for therapeutic applications. Realising this potential requires further characterization of the diversity of biomolecules present in tissue-specific ECMs and an evaluation of their role as regulatory cues for regenerative medicine applications. The goal of this study was to identify specific soluble factors within the ECM of articular cartilage (AC) and growth plate (GP) that may impart chondro-inductivity or osteo-inductivity respectively. To this end, the significantly different proteins between both matrisomes were searched against the STRING database platform, from which C-type lectin domain family-11 member-A (CLEC11A) and S100 calcium-binding protein-A10 (S100A10) were identified as potential candidates for supporting osteogenesis, and Gremlin-1 (GREM1) and TGF-β induced gene human clone-3 (βIGH3) were identified as potential candidates for supporting stable chondrogenesis. Stimulation of chondrogenically-primed bone marrow-derived stem cells (BMSCs) with the AC-specific proteins GREM1 and βIGH3 had no noticeable effect on the deposition of collagen-II, a marker of chondrogenesis, but appeared to suppress the production of the hypertrophic marker collagen-X, particularly for higher concentrations of GREM1. Stimulation with GREM1 was also found to suppress the direct osteoblastic differentiation of BMSCs. In contrast, stimulation with the GP-specific factors CLEC11A and S100A10 was found to enhance osteogenesis of BMSCs, increasing the levels of mineralization, particularly for higher concentration of CLEC11A. Together these results demonstrate that AC- and GP-specific proteins may play a key role in developing novel strategies for engineering phenotypically stable articular cartilage or enhancing the regeneration of critically-sized bone defects.


## 1. Introduction

The repair of critically-sized cartilage, bone and osteochondral defects remains a significant clinical challenge [1]. Putative tissue engineering (TE) strategies to treat such defects require the identification of biomaterial scaffolds and regulatory cues to actively modulate the adhesion, proliferation, migration and differentiation of cells [2] that are either recruited into and/or seeded onto such constructs. Extracellular matrix (ECM) derived scaffolds that maintain native tissue complexity have been under investigation for more than 25 years [3] and during that time have been implanted into millions of patients [4]. Most commercial ECM scaffolds are derived from soft tissues (dermis, urinary bladder, small intestinal submucosa), although they have been used in the repair of many different tissue types [5]. In recent years it has become increasingly clear that the ECM of a specific tissue is particularly supportive of the phenotype of specialized cells resident within that tissue [6–13]. For example, ECM fractions of tendon and cartilage have been shown to enhance the tissue-specific differentiation of bone marrow-derived stem cells

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(BMSCs) [6]. This suggests that tissue-specific ECM proteins can play a key role in regulating the fate of BMSCs and potentially be utilized for therapeutic applications. Realising this potential will require further characterization of the diversity of biomolecules present in tissue-specific ECMs and evaluation of the role of specific proteins as regulatory cues for TE applications.

The ECM of musculoskeletal tissues is commonly used to produce biomaterials and scaffolds for orthopaedic applications [14,15]. In a previous study [13], we compared the ECM proteome (the *Matrisome* [16]) of two cartilaginous tissues, the growth plate (GP; region of developing long bones where new bone growth takes place) and articular cartilage (AC), and demonstrated the potential of scaffolds derived from such tissues for bone, cartilage and osteochondral TE. Building on this work, the goal of this study was first to identify specific soluble factors within this protein list that may impart chondroinductivity or osteoinductivity to the ECM of AC and GP respectively. To this end, the significantly different proteins between both matrisomes were searched against the STRING [17] database. STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*) is a database of known and computationally predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations. Protein-protein interaction networks are important to understand cellular processes. The predicted networks act as a filtering system that can provide an intuitive platform for functional annotations and suggest new directions for future experimental research. The second objective focused on assessing the influence of two AC-specific and two GP-specific proteins on both chondrogenesis and osteogenesis of BMSCs, respectively. We were particularly interested in identifying AC-specific proteins that may play a role in supressing chondrocyte hypertrophy and progression along an endochondral pathway, a key challenge in BMSC based cartilage TE [18,19], and alternatively GP-specific proteins that could potentially be used for bone TE. To model chondrogenesis, BMSCs were cultured in a pellet model and stimulated with chondrogenic medium supplemented with AC-specific proteins. Osteogenesis was assessed using BMSCs cultured in a monolayer model with osteogenic medium supplemented with GP-specific proteins.

## 2. Methods

### 2.1. STRING database analysis

The protein list searched against the STRING database was obtained from a previous study [13]. Briefly, AC and GP-ECMs proteome identification was done by high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). The MS/MS-derived raw data was analysed using Maxquant [20]. Peptides that passed the 1% false discovery rate (FDR) threshold were used for label free quantification (LFQ) protein identification. Proteins identified by Maxquant were processed with Perseus [21] in order to elucidate differences across the two tissues. The significantly different proteins from each tissue (namely tissue-specific) were searched against the matrisome database to focus only on the proteins of the ECM. In this study, the obtained matrisome protein list was then searched against the STRING database. STRING was used to create protein-protein interaction networks and cluster proteins into subgroups in order to identify enriched biological pathways and functional annotations, allowing the identification of key molecules of interest. The associated information for each protein in STRING was then manually curated to select proteins of interest. An extended literature review of the four proteins selected can be found in the Appendix.

### 2.2. Bone marrow-derived stem cell (BMSCs) isolation

BMSCs were harvested from the femora of porcine donors (4-months-old) within 3 h of sacrifice as previously described [22]. BMSCs were used at passage 2 for each experiment after expansion in XPAN medium: high-glucose Dulbecco's modified Eagle's medium (DMEM)+GlutaMAX™, supplemented with 10% foetal bovine serum.

All media were supplemented with 100U/ml Penicillin, 100U/ml Streptomycin and 0.25 μg/ml amphotericin B (all Gibco®).

### 2.3. Analysis of AC-specific proteins using an in vitro pellet model in chondrogenesis

Pellets were formed by centrifuging 250,000 BMSCs in a 1.5 ml microtube at 650 g for 5 min. The pellets were cultured in normoxia (20% $pO_2$) at 37 °C, for 21 days in chondrogenic medium (CM): DMEM, 1 mM sodium pyruvate, 350 μM L-proline, 1.5 mg/ml bovine serum albumin, 1 nM dexamethasone, 300 μM ascorbic acid, 17 μM linolenic acid, 10 ng/ml transforming growth factor-β3 (TGF-β3, R&D Systems®), 1X insulin-transferrin-selenium. Experimental groups were designed as followed: CM, CM+20 or 200 ng/ml GREM1, CM+20 or 200 ng/ml βIGH3. Media was replenished twice per week.

Samples at days 2 and 21 ($n \geq 2$) were fixed in 4% paraformaldehyde, dehydrated and wax embedded to allow serial slicing (6 μm). Staining was performed using 1% alcian blue-8GX in 0.1 M HCl and 0.1% picro-sirius red for visualization of sulphated-glycosaminoglycan (sGAG) and collagen deposition, respectively. Collagen-II and X deposition was evaluated using immunohistochemistry. Primary antibody: 1:100 anti-collagen-II (ab3092, Abcam®) or 1:100 anti-collagen-X (ab49945, Abcam®). Biochemical analysis was used to supplement histological findings by measuring DNA (Hoescht assay), sGAG (DMMB assay, Blyscan, Biocolor) and hydroxyproline (Chloramine-T assay, indirect collagen measure [23]) content in papain digested samples ($n \geq 3$), as previously described [24].

### 2.4. Influence of tissue-specific proteins on osteogenesis of BMSCs using a monolayer culture model

BMSCs were seeded onto 24 well plates at $10^3$ cells/cm$^2$ and allowed to proliferate for 24 h in XPAN. Then, cells were cultured for 21 days in the following groups: Osteogenic medium as a positive control (OM): XPAN, 5 mM β-glycerophosphate, 1 nM dexamethasone, 0.09 mM ascorbic acid. Negative control (-OM): OM without β-glycerophosphate. -OM or OM were supplemented with 10, 50, 100 ng/ml of CLEC11A or S100A10.

After 21 days of culture, monolayers were stained for calcium analysis with 1% alizarin red solution. Images were taken with a brightfield microscope. Semi-quantitative analysis of mineralization was performed by analysis of the positively stained area with Photoshop-CS6.

### 2.5. Statistical analysis

Results are presented as mean ± standard deviation. Graphical results and statistical analysis were performed with GraphPad Prism. Experimental groups were analysed for significant differences using Tukey's test to compare different conditions. Statistically significant changes are marked as * = $p \leq 0.05$; ** = $p \leq 0.01$.

## 3. Results

### 3.1. Matrisome and STRING can be used as filters to identify proteins of interest

A protein list containing the significantly different proteins between AC and GP ECMs, identified in a previous study [13], was used to run a matrisome analysis. In this analysis, the number of proteins was reduced from 242 to 51 for AC (blue circle in Fig. 1a) and from 548 to 60 for GP (red). The proteins were divided into the following matrisome-subcategories: collagen, ECM glycoproteins, proteoglycans (core matrisome), ECM-affiliated, ECM regulators and secreted factors (matrisome-associated); illustrated using a heat map (Fig. 1b). The AC tissue contained more collagens, proteoglycans and ECM glycoproteins than the GP. In contrast, the GP tissue contained more ECM regulators, ECM-affiliated and secreted factors when compared to the AC tissue. Only 91 proteins of the 111 (51 in AC; 60 in GP) tissue-specific proteins were found in the STRING database. The generated protein network (Fig. 1c) helped, firstly, to spatially arrange the proteins from each tissue (blue for AC and red for GP) so as to predict interactions between them. Associations are meant to be meaningful, *i.e.,* proteins jointly contribute to a shared function; this does not necessarily mean they are physically interacting with each other. The STRING database also provides brief information on the input proteins that was manually reviewed by searching for keywords such as 'collagen', 'chondrogenesis', 'bone' and 'mineralization'. These two features facilitated identification of the following 4 proteins (highlighted in Fig. 1c): GREM1, βIGH3, CLEC11A, S100A10. For example, part of the information found in STRING regarding GREM1 was "inhibits BMP2-mediated differentiation of osteoblasts", suggesting that it may play a role in supressing hypertrophy of BMSCs. Information on βIGH3 was: "Plays a role in cell adhesion and cell-collagen interactions". In addition, the network linked it to heparan sulfate (HSPG2), which is essential in cartilage development [25]. Information on CLEC11A included "promotes osteogenesis", making it a potential candidate for enhancing osteogenesis of BMSCs. Information on S100A10 included "regulator of ANXA2" and the network linked it to ANXA5, which have been shown to regulate matrix vesicle-mediated mineralization [26].

### 3.2. AC-specific proteins can modulate chondrogenesis and hypertrophy of BMSCs

Having identified two AC-specific proteins of interest (GREM1 and βIGH3), we next sought to study their effect on chondrogenesis and hypertrophy of BMSCs. BMSC pellets were cultured for 21 days in chondrogenic medium (CM) or CM additionally supplemented with the AC-specific factors GREM1 or βIGH3 at two different concentrations (20 or 200 ng/ml). All pellets stained positive for alcian blue and picrosirius red, indicative of sGAG and collagen deposition, respectively (Fig. 2a). The most robust staining for sGAG deposition was observed in pellets supplemented with βIGH3. More intense staining for collagen was observed in pellets only supplemented with standard CM. To confirm the development of hyaline cartilage, the deposition of collagen-II and X within the pellets was next analysed by immunohistochemistry. Robust collagen-II deposition was observed in all groups. Pellets supplemented with standard CM alone also stained strongly for collagen-X deposition, with lower levels observed in the groups stimulated with βIGH3. Pellets stimulated with GREM1 stained weakly for collagen-X. Quantitative biochemical analysis was undertaken to supplement the histological results, which revealed similar trends in terms of tissue deposition (Fig. 2b), although no statistically significant differences in DNA, sGAG and hydroxyproline (HxP, indirect measure of collagen) levels were observed between the groups.

As both GREM1 or βIGH3 appeared to suppress the synthesis of collagen-X, a marker of chondrocyte hypertrophy and progression along the endochondral pathway, we next sough to assess the effect of these AC-specific proteins on direct osteogenesis of BMSCs. BMSCs were grown in monolayer culture and supplemented with osteogenic media (OM) with or without the highest concentration (200 ng/ml) of either GREM1 or βIGH3. After 14 days of culture, alizarin red staining revealed that βIGH3 stimulation had no noticeable effect on mineralization, while it was dramatically supressed by GREM1 stimulation (Fig. 2c). Image analysis provided semi-quantification of these observations, demonstrating significant differences between standard OM and OM+βIGH3 cultures compared to the negative control (-OM) and OM + GREM1 group.

### 3.3. GP-specific proteins enhance osteogenesis of BMSCs

BMSCs were cultured at a density of $10^3$ cells/cm$^2$ in -OM (Fig. 3a) or OM (Fig. 3b) for 2 weeks. Samples were supplemented with either 10, 50, 100 ng/ml of CLEC11A or S100A10. Alizarin red staining was performed on the samples after 2 weeks to analyse calcium deposition. There was little evidence of mineral deposition in BMSCs cultured in the negative control medium. Semi-quantitative analysis of stained samples demonstrated a dose-dependent increase in mineral deposition for all experimental groups. The highest values were observed following stimulation with the CLEC11A protein (Fig. 3c), although significant increase in mineralization was also observed with S100A10 stimulation.

## 4. Discussion

The complexity and diversity of biomolecules present in tissue-specific ECMs are not yet fully characterized. This study firstly focused on the proteomic characterization of the soluble ECM of two different musculoskeletal tissues, articular cartilage and growth plate, which we have previously demonstrated can promote differential tissue deposition by BMSCs when processed into porous scaffolds [13,24,27]. This can be directly correlated to the unique composition of AC and GP ECM, which was characterized using an in-depth analysis of the less abundant non-collagenous proteins in these tissues. From the specific proteins identified by this analysis, we found that the AC-specific protein GREM1 could be used to promote the development of phenotypically stable articular cartilage, while the GP-specific protein CLEC11A was particularly potent in promoting osteogenesis of BMSCs.

The combination of matrisome and STRING databases was used to identify functional annotations and individual protein-protein interactions that could play a key role in cartilage and bone regeneration. Among the many different associated biological processes, we focused on those perceived to be relevant to osteochondral TE, and as expected, observed that AC and GP ECMs contain proteins associated with chondrogenesis and osteogenesis, respectively. After reviewing the information gathered from STRING, four soluble factors were chosen for further analysis to determine their effect on either chondrogenesis or osteogenesis of BMSCs. These proteins were GREM1 (ECM-affiliated) and βIGH3 (ECM glycoprotein) from AC, and CLEC11A (ECM-affiliated) and S100A10 (secreted factor) from GP. Previous mass spectrometry studies of AC have also revealed the presence of GREM1 [28] and TGFβI/βIGH3 [29] proteins at different depths within human AC and other cartilaginous tissues [30]. The detection of S100A10 [26] and osteolectin/CLEC11A [31] in the GP ECM is in agreement with past studies, confirming that hypertrophic chondrocytes resident in the GP express such proteins.

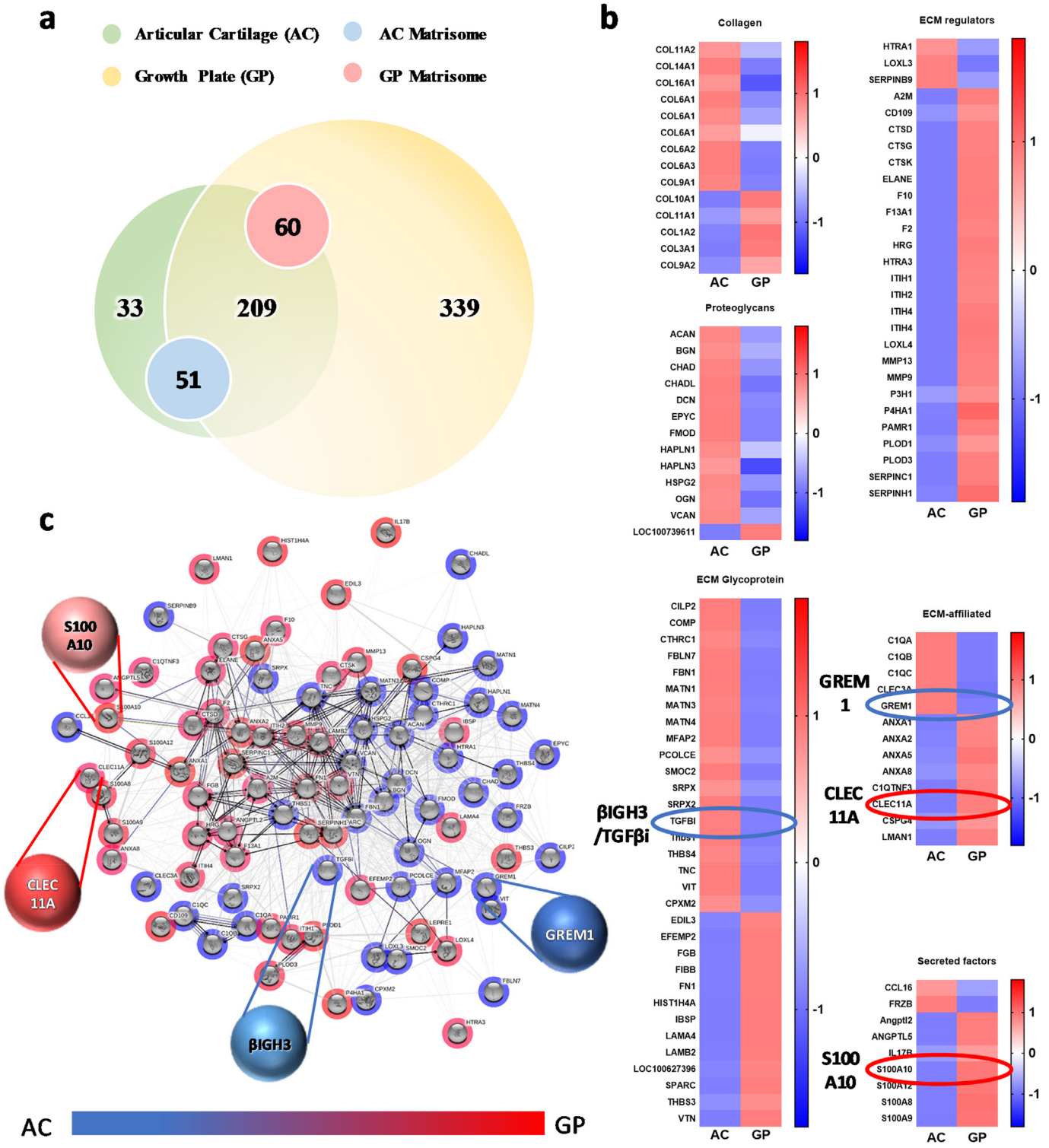


**Fig. 1.** Refinement of matrisome analysis from our previous study [13]. **a**, Venn diagram showing the tissue-specific proteins (AC: dark green; GP: yellow), the shared proteins (light green) and significantly different proteins found in the matrisome of each tissue (AC-matrisome: blue; GP-matrisome: red). **b**, STRING network of all significantly different proteins found in the matrisome, including a payload to differentiate each tissue (AC:blue; GP: red). Four proteins of interest have been highlighted with large spheres. **c**, Heat map of the z-score statistical measure of the significantly different proteins found in the 6 matrisome subcategories: collagen, proteoglycans, ECM glycoproteins, ECM-regulators, ECM-affiliated and secreted factors. The four proteins of interest have been highlighted with a circle. Significantly different proteins are based on LFQ intensities within the two tissues (1%FDR). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

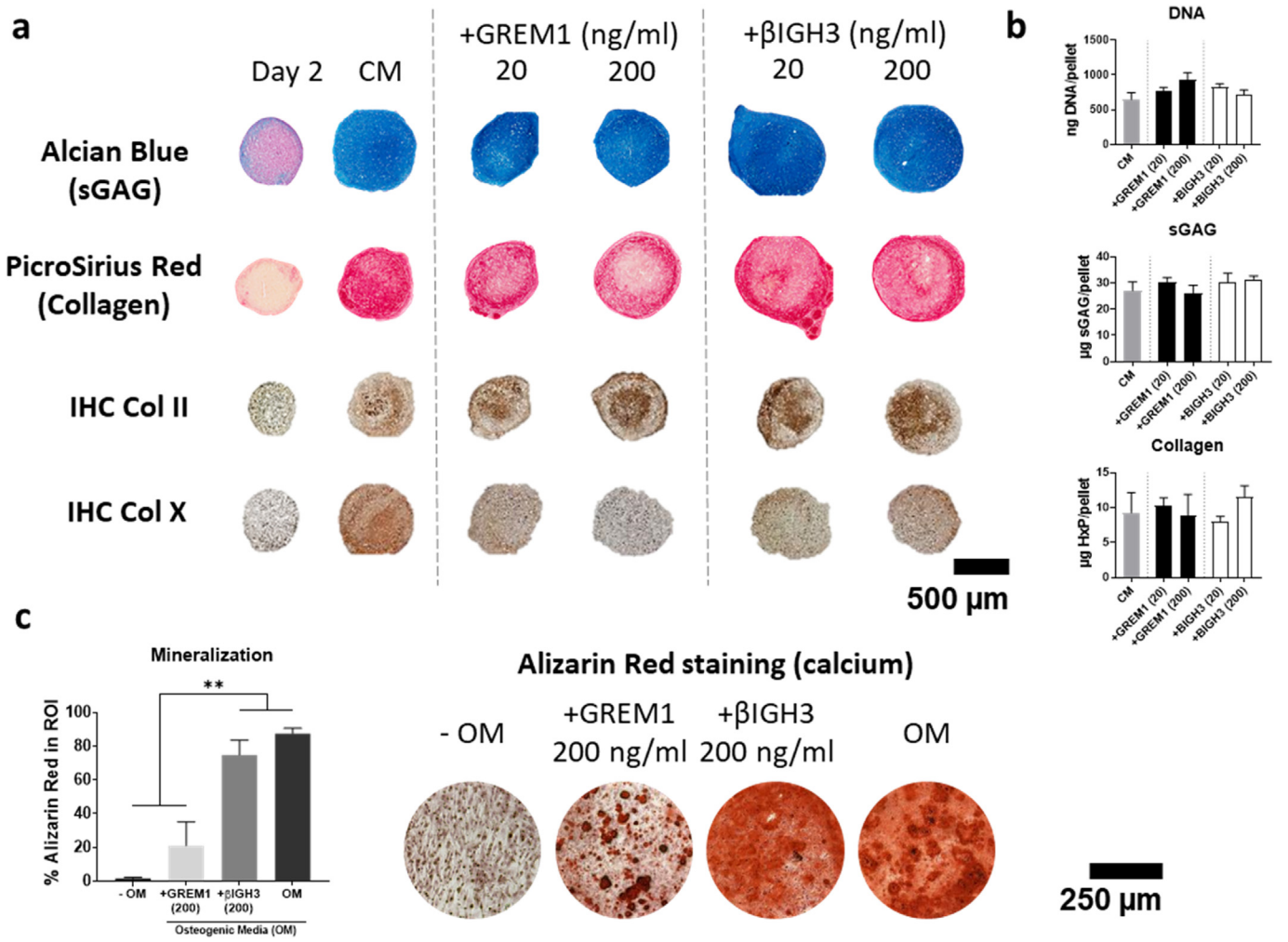


**Fig. 2.** Effect of AC-specific proteins in BMSC chondrogenesis and osteogenesis. **a**, Histological analysis of pellets cultured for 21 days in chondrogenic media showing alcian blue for sGAG staining, picrosirius red for collagen staining and immunohistochemistry (IHC) for the chondrogenic marker collagen-II and for the hypertrophic marker collagen-X. Experimental groups are: day 2 control, standard chondrogenic medium (CM), CM+20 or 200 ng/ml of either GREM1 or βIGH3 ($n \geq 2$). **b**, Biochemical analysis showing DNA, sGAG and HxP levels per pellet for all groups after 21 days of culture ($n \geq 4$). **c**, Left: alizarin red staining representative images of monolayer model ($n \geq 2$). Experimental groups: osteogenic media without β-glycerophosphate (-OM) and osteogenic media (OM) +/- 200 ng/ml of either GREM1 or βIGH3 for 14 days. Right: semi-quantification analysis of the amount of red staining in the region of interest (ROI) of all groups in percentage (**$p \leq 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Stimulating BMSCs undergoing chondrogenesis with AC-specific proteins led to the development of a more phenotypically stable hyaline-like cartilage, as evident by a reduction in the hypertrophic marker collagen-X. This is in agreement with previous studies showing similar results for GREM1 in pellet culture of BMSCs [28]. In addition, GREM1 overexpression has also been shown to suppress BMP-mediated bone formation [32]. We also demonstrated for the first time that the AC-protein, βIGH3, is also capable of supressing hypertrophy without suppressing overall levels of chondrogenesis in BMSCs, with βIGH3 stimulated pellets staining intensely for sGAGs and collagen-II. Previous studies have highlighted that recombinant βIGH3 inhibits the mineralization of hypertrophic chondrocytes [29], while the biomechanical regulation of chondrocyte hypertrophy has also been associated with increased βIGH3 expression with the application of strain [33]. In this study, GREM1, but not βIGH3, was able to supress β-glycerophosphate mediated mineralization during monolayer culture of BMSCs. This demonstrates that AC contains multiple proteins that play a role in supressing endochondral ossification of the tissue through different pathways, pointing to a complex regulatory mechanism. Due to the more potent influence of GREM1 on reducing hypertrophy and mineralization in different *in vitro* models, future studies from our lab will explore its potential to engineer phenotypically stable articular cartilage *in vivo*.

The supplementation of GP-specific proteins enhanced osteogenesis of BMSCs in a monolayer culture model. In the absence of β-glycerophosphate, neither CLEC11A nor S100A10 were observed to promote osteogenesis. These results suggest that, at the dosages explored in this study, that these proteins in isolation do not promote osteogenesis, however, they can act in combination with osteogenic factors to enhance the osteogenic phenotype. Due to their role in the maintenance of the adult skeleton [31] and vesicle-derived matrix ossification (mature matrix) [34], their effect may be more evident in different *in vitro* and *in vivo* models of osteogenesis and endochondral ossification. CLEC11A was particularly potent in promoting osteogenesis of BMSCs, which agrees with a recent study demonstrating that recombinant CLEC11A promoted osteogenesis of stromal cells in culture and increased bone mass in osteoporotic mice *in vivo* [31].

Taken together, the results of this study demonstrated that

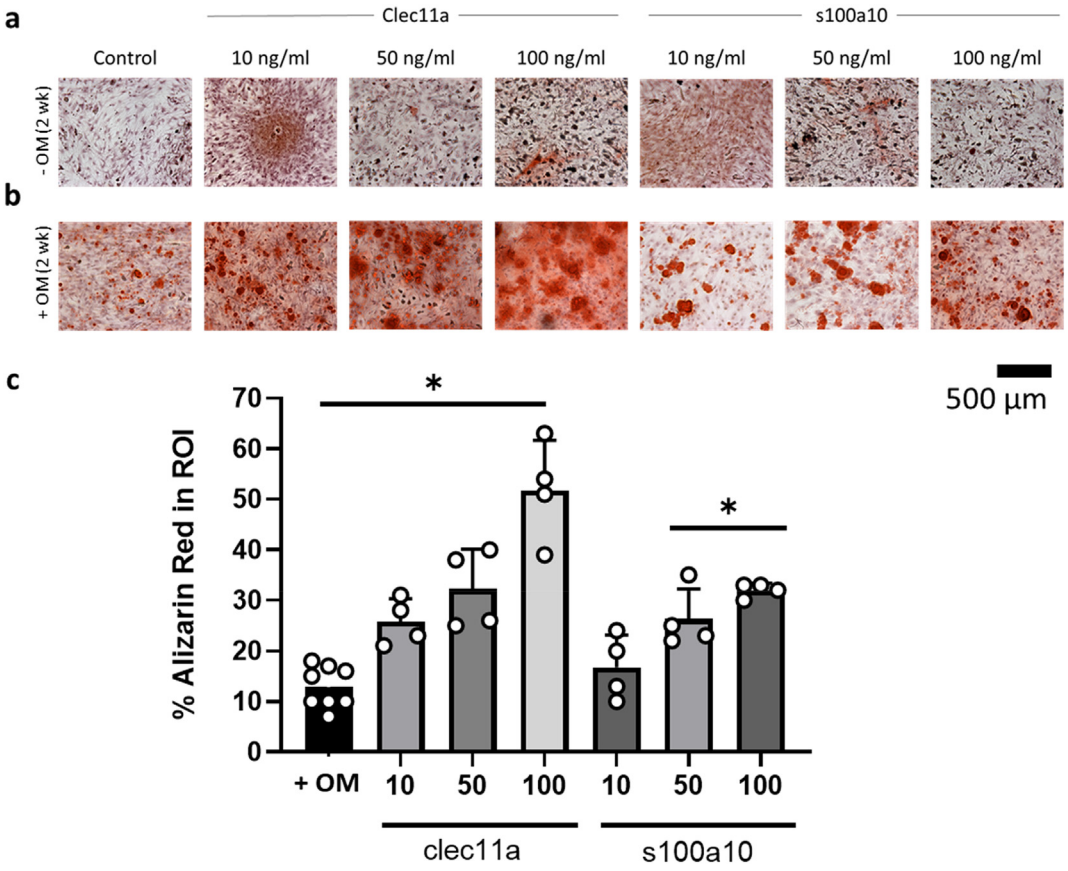


**Fig. 3.** Effect of GP-specific proteins on BMSC osteogenesis cultured in monolayer model. **a**, Alizarin red staining of wells containing cells after 14 days in negative control medium (-OM) supplemented with either CLEC11A or S100A10 at three different concentrations: 10, 50 and 100 ng/ml **b**, Alizarin red staining of the same groups with positive control medium (OM) after 14 days of culture. **c**, Semi-quantification of red staining in **b** (*$p \leq 0.05$; $n \geq 4$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

bioinformatic approaches combining Matrisome and STRING databases can help elucidate the complexity and the diversity or uniformity of proteins across different tissues and can be useful for identifying tissue-specific growth factors for tissue engineering and regenerative medicine applications. It was demonstrated that AC- and GP-specific proteins may play a key role in developing novel strategies for engineering phenotypically stable articular cartilage or enhancing the regeneration of critically-sized bone defects.

## Declaration of competing interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.05.074.

## References

[1] D.J. Huey, J.C. Hu, K. Athanasiou, Unlike bone, cartilage regeneration remains elusive, Science 338 (2012) 917–921, https://doi.org/10.1126/science.1222454.
[2] E.A. Makris, A.H. Gomoll, K.N. Malizos, J.C. Hu, K.A. Athanasiou, Repair and tissue engineering techniques for articular cartilage, Nat. Rev. Rheumatol. 11 (2014) 1–14, https://doi.org/10.1038/nrrheum.2014.157.
[3] S.F. Badylak, G.C. Lantz, A. Coffey, L. a Geddes, Small intestinal submucosa as a large diameter vascular graft in the dog, J. Surg. Res. 47 (1989) 74–80, https://doi.org/10.1016/0022-4804(89)90050-4.
[4] R.M. Nerem, Regenerative medicine: the emergence of an industry, J. R. Soc. Interface 7 (2010) S771–S775, https://doi.org/10.1098/rsif.2010.0348.focus.
[5] S.F. Badylak, The extracellular matrix as a biologic scaffold material, Biomaterials 28 (2007) 3587–3593, https://doi.org/10.1016/j.biomaterials.2007.04.043.
[6] B.B. Rothrauff, G. Yang, R.S. Tuan, Tissue-specific bioactivity of soluble tendon-derived and cartilage-derived extracellular matrices on adult mesenchymal stem cells, Stem Cell Res. Ther. 8 (2017) 1–17, https://doi.org/10.1186/s13287-017-0580-8.
[7] J.L. Dziki, D.S. Wang, C. Pineda, B.M. Sicari, T. Rausch, S.F. Badylak, Solubilized extracellular matrix bioscaffolds derived from diverse source tissues differentially influence macrophage phenotype, J. Biomed. Mater. Res. 105 (2017) 138–147, https://doi.org/10.1002/jbm.a.35894.
[8] B.B. Rothrauff, K. Shimomura, R. Gottardi, P.G. Alexander, R.S. Tuan, Anatomical region-dependent enhancement of 3-dimensional chondrogenic differentiation of human mesenchymal stem cells by soluble meniscus extracellular matrix, Acta Biomater. 49 (2016) 140–151, https://doi.org/10.1016/j.actbio.2016.11.046.
[9] E.J. Sheehy, D.J. Kelly, F.J. O'Brien, Biomaterial-based endochondral bone regeneration: a shift from traditional tissue engineering paradigms to developmentally inspired strategies, Mater. Today Bio. 3 (2019) 1–13, https://doi.org/10.1016/j.mtbio.2019.100009.
[10] D. Zhang, D. He, G. Wei, Y. Zhang, Y. Zhang, H. Yi, Z. Wang, R. Wu, Y. Hu, J. Deng, T. Criswell, J. Yoo, Y. Zhou, A. Atala, S. Wei, Tissue-specific extracellular matrix enhances skeletal muscle precursor cell expansion and differentiation

for potential application in cell therapy, Tissue Eng. 23 (2017) 784–794, https://doi.org/10.1089/ten.TEA.2016.0489.
[11] L. Li, G. Liu, P. Timashev, X.S. Sun, T. Criswell, A. Atala, Y. Zhang, Biofabrication of tissue-specific extracellular matrix proteins to enhance the expansion and differentiation of skeletal muscle progenitor cells, Appl. Phys. Rev. 6 (2019) 1–6, https://doi.org/10.1063/1.5088726.
[12] D. Lam, H.A. Enright, J. Cadena, S.K.G. Peters, A.P. Sales, J.J. Osburn, D.A. Soscia, K.S. Kulp, E.K. Wheeler, N.O. Fischer, Tissue-specific extracellular matrix accelerates the formation of neural networks and communities in a neuron-glia co-culture on a multi-electrode array, Sci. Rep. 9 (2019) 1–15, https://doi.org/10.1038/s41598-019-40128-1.
[13] G.M. Cunniffe, P.J. Díaz-Payno, E.J. Sheehy, S.E. Critchley, H.V. Almeida, P. Pitacco, S.F. Carroll, O.R. Mahon, A. Dunne, T.J. Levingstone, C.J. Moran, R.T. Brady, F.J. O'Brien, P.A.J. Brama, D.J. Kelly, Tissue-specific extracellular matrix scaffolds for the regeneration of spatially complex musculoskeletal tissues, Biomaterials 188 (2019) 63–73, https://doi.org/10.1016/j.biomaterials.2018.09.044.
[14] P. Nooeaid, V. Salih, J.P. Beier, A.R. Boccaccini, Osteochondral tissue engineering: scaffolds, stem cells and applications, J. Cell Mol. Med. 16 (2012) 2247–2270, https://doi.org/10.1111/j.1582-4934.2012.01571.x.
[15] D.C. Browe, O.R. Mahon, P.J. Díaz-Payno, N. Cassidy, I. Dudurych, A. Dunne, C.T. Buckley, D.J. Kelly, Glyoxal cross-linking of solubilized extracellular matrix to produce highly porous, elastic, and chondro-permissive scaffolds for orthopedic tissue engineering, J. Biomed. Mater. Res. 107 (2019) 2222–2234, https://doi.org/10.1002/jbm.a.36731.
[16] R.O. Hynes, A. Naba, Overview of the matrisome-An inventory of extracellular matrix constituents and functions, Cold Spring Harb. Perspect. Biol. 4 (2012) 1–16, https://doi.org/10.1101/cshperspect.a004903.
[17] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, K.P. Tsafou, M. Kuhn, P. Bork, L.J. Jensen, C. Von Mering, STRING v10: protein-protein interaction networks, integrated over the tree of life, Nucleic Acids Res. 43 (2015) D447–D452, https://doi.org/10.1093/nar/gku1003.
[18] R. Narcisi, M.A. Cleary, P.A.J. Brama, M.J. Hoogduijn, N. Tüysüz, D. Ten Berge, G.J.V.M. Van Osch, Long-term expansion, enhanced chondrogenic potential, and suppression of endochondral ossification of adult human MSCs via WNT signaling modulation, Stem Cell Rep. 4 (2015) 459–472, https://doi.org/10.1016/j.stemcr.2015.01.017.
[19] D.C. Browe, C.M. Coleman, F.P. Barry, S.J. Elliman, Hypoxia activates the PTHrP–MEF2C pathway to attenuate hypertrophy in mesenchymal stem cell derived cartilage, Sci. Rep. 9 (2019) 13274, https://doi.org/10.1038/s41598-019-49499-x.
[20] S. Tyanova, T. Temu, J. Cox, The MaxQuant computational platform for mass spectrometry-based shotgun proteomics, Nat. Protoc. 11 (2016) 2301–2319, https://doi.org/10.1038/nprot.2016.136.
[21] S. Tyanova, T. Temu, P. Sinitcyn, A. Carlson, M.Y. Hein, T. Geiger, M. Mann, J. Cox, The Perseus computational platform for comprehensive analysis of (prote)omics data, Nat. Methods 13 (2016) 731–740, https://doi.org/10.1038/nmeth.3901.
[22] S.D. Thorpe, C.T. Buckley, T. Vinardell, F.J. O'Brien, V.A. Campbell, D.J. Kelly, The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-β3 induced chondrogenic differentiation, Ann. Biomed. Eng. 38 (2010) 2896–2909, https://doi.org/10.1007/s10439-010-0059-6.
[23] N.Y. Ignat'eva, N.A. Danilov, S.V. Averkiev, M.V. Obrezkova, V.V. Lunin, E.N. Sobol', Determination of hydroxyproline in tissues and the evaluation of the collagen content of the tissues, J. Anal. Chem. 62 (2007) 51–57, https://doi.org/10.1134/S106193480701011X.
[24] G.M. Cunniffe, P.J. Díaz-Payno, J.S. Ramey, O.R. Mahon, A. Dunne, E.M. Thompson, F.J. O'Brien, D.J. Kelly, Growth plate extracellular matrix-derived scaffolds for large bone defect healing, Eur. Cell. Mater. 33 (2017) 130–142, https://doi.org/10.22203/eCM.v033a10.
[25] R. Sadatsuki, H. Kaneko, M. Kinoshita, I. Futami, R. Nonaka, K.L. Culley, M. Otero, S. Hada, M.B. Goldring, Y. Yamada, K. Kaneko, E. Arikawa-Hirasawa, M. Ishijima, Perlecan is required for the chondrogenic differentiation of synovial mesenchymal cells through regulation of Sox9 gene expression, J. Orthop. Res. 35 (2017) 837–846, https://doi.org/10.1002/jor.23318.
[26] A. Cmoch, A. Strzelecka-Kiliszek, M. Palczewska, P. Groves, S. Pikula, S100A4 and S100A10 proteins as regulators of matrix vesicle mediated mineralization of osteoblast-like cells, Bone 50 (2012) S69–S70, https://doi.org/10.1016/j.bone.2012.02.196.
[27] H.V. Almeida, Y. Liu, G.M. Cunniffe, K.J. Mulhall, A. Matsiko, C.T. Buckley, F.J. O'Brien, D.J. Kelly, Controlled release of transforming growth factor-β3 from cartilage-extra-cellular-matrix-derived scaffolds to promote chondrogenesis of human-joint-tissue-derived stem cells, Acta Biomater. 10 (2014) 4400–4409, https://doi.org/10.1016/j.actbio.2014.05.030.
[28] J.C.H. Leijten, J. Emons, C. Sticht, S. Van Gool, E. Decker, A. Uitterlinden, G. Rappold, A. Hofman, F. Rivadeneira, S. Scherjon, J.M. Wit, J. Van Meurs, C.A. Van Blitterswijk, M. Karperien, Gremlin 1, frizzled-related protein, and dkk-1 are key regulators of human articular cartilage homeostasis, Arthritis Rheum. 64 (2012) 3302–3312, https://doi.org/10.1002/art.34535.
[29] S. Ohno, T. Doi, S. Tsutsumi, Y. Okada, K. Yoneno, Y. Kato, K. Tanne, RGD-CAP (βig-h3) is expressed in precartilage condensation and in prehypertrophic chondrocytes during cartilage development, Biochim. Biophys. Acta Gen. Subj. 1572 (2002) 114–122, https://doi.org/10.1016/S0304-4165(02)00286-6.
[30] P. Önnerfjord, A. Khabut, F.P. Reinholt, O. Svensson, D. Heinegård, Quantitative proteomic analysis of eight cartilaginous tissues reveals characteristic differences as well as similarities between subgroups, J. Biol. Chem. 287 (2012), https://doi.org/10.1074/jbc.M111.298968, 18913–24.
[31] R. Yue, B. Shen, S.J. Morrison, Clec11a/osteolectin is an osteogenic growth factor that promotes the maintenance of the adult skeleton, Elife 5 (2016) 1–27, https://doi.org/10.7554/eLife.18782.
[32] E. Gazzerro, R.C. Pereira, V. Jorgetti, S. Olson, A.N. Economides, E. Canalis, Skeletal overexpression of gremlin impairs bone formation and causes osteopenia, Endocrinology 146 (2005) 655–665, https://doi.org/10.1210/en.2004-0766.
[33] S. Ohno, N. Tanaka, M. Ueki, K. Honda, K. Tanimoto, K. Yoneno, M. Ohno-Nakahara, K. Fujimoto, Y. Kato, K. Tanne, Mechanical regulation of terminal chondrocyte differentiation via RGD-CAP/βig-h3 induced by TGF-β, Connect. Tissue Res. 46 (2005) 227–234, https://doi.org/10.1080/03008200500346111.
[34] A. Cmoch, A. Strzelecka-Kiliszek, M. Palczewska, P. Groves, S. Pikula, Matrix vesicles isolated from mineralization-competent Saos-2 cells are selectively enriched with annexins and S100 proteins, Biochem. Biophys. Res. Commun. 412 (2011) 683–687, https://doi.org/10.1016/j.bbrc.2011.08.025.