Growth Hormone & IGF Research 42–43 (2018) 14–21

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

journal homepage: www.elsevier.com/locate/ghir

A characteristic signature of insulin-like growth factor (IGF) axis expression during osteogenic differentiation of human dental pulp cells (hDPCs): Potential co-ordinated regulation of IGF action

Hasanain Al-Khafaji[a], Pernille R. Noer[d], Hanna Alkharobi[b], Aishah Alhodhodi[a], Josephine Meade[a], Reem El-Gendy[a,c], Claus Oxvig[d], James Beattie[a,*]

[a] Division of Oral Biology, Leeds School of Dentistry, Level 7 Wellcome Trust Brenner Building, University of Leeds, St James University Hospital, Leeds, United Kingdom
[b] Department of Oral Biology, Dental College, King AbdulAziz University, Jeddah, Saudi Arabia
[c] Department of Oral Pathology, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt
[d] Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10C, 8000 Aarhus C, Denmark

ABSTRACT

The IGF axis is represented by two growth factors (IGF1 and IGF2), two cognate cell surface receptors (IGF1R and IGF2R), six soluble high affinity IGF binding proteins (IGFBP1-6) and several IGFBP proteases. IGF1 and IGF2 are present at high concentrations in bone and play a crucial role in the maintenance and differentiation of both foetal and adult skeleton. In order to understand the role of the IGF axis in bone and other tissues it is necessary to profile the expression and activity of all genes in the axis together with the activity of relevant ancillary proteins (including IGFBP proteases). In the current report we used differentiating human dental pulp cells (hDPC) to examine the expression and activity of the IGF axis during osteogenic differentiation of these cells. We found that, with the exception of IGF1 and IGFBP1, all components of the IGF axis are expressed in hDPCs. IGFBP-4 is the most abundantly expressed IGFBP species at both mRNA and protein levels under both basal and osteogenic conditions. Although we found no difference in IGFBP-4 expression under osteogenic conditions, we report increased expression and activity of pregnancy associated plasma protein-A (PAPP-A - an IGFBP-4 proteinase) leading to increased IGFBP-4 proteolysis in differentiating cell cultures. Further to this we report increased expression of IGF-2 (an activator of PAPP-A), and decreased expression of stanniocalcin-2 (STC2- a recently discovered inhibitor of PAPP-A) under osteogenic conditions. We also demonstrate that STC2 and PAPP-A are able to form complexes in hDPC conditioned medium indicating the potential for regulation of IGFBP-4 proteolysis through this mechanism. We suggest that these changes in the expression and activity of the IGF axis may represent part of an osteogenic signature characteristic of differentiating hDPCs.

1. Introduction

The insulin-like growth factor (IGF) axis comprises two polypeptide growth factors (IGF1 and IGF2), two cell surface receptors (IGF1R and IGF2R) and six soluble high-affinity IGF binding proteins (IGFBP-1-6). The IGF axis plays a crucial role in the development and maintenance of the mineralised skeleton and disruption of the IGF1 or IGF1R gene compromises skeletal growth in rodents and humans [1, 2]. IGFs are the most abundant growth factors in bone tissue [3] and in addition to an anabolic role in mature bone, the IGF axis also regulates osteoblast and osteoclast differentiation in developing bone tissue, controlling the balance between bone accretion and resorption which occurs throughout life [4–6]. Accordingly, conditional knockout of IGF1 or IGF1R in osteoblasts or mature osteocytes compromises bone development and impairs osteogenic differentiation of mesenchymal stem cells [7, 8]. In bone and other tissues the activity of IGFs is regulated by the presence of high affinity IGFBPs [9] and we have previously demonstrated that IGFBP-2 and IGFBP-3 regulate exogenous IGF-1 stimulation of osteogenic differentiation in cultures of human dental pulp cells (hDPCs)·Therefore increased IGFBP-2 expression in these cells is associated with enhanced IGF-1 action while expression of the inhibitory IGFBP-3 is decreased during hDPC differentiation [10]. Although this

Abbreviations: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; PAPP-A, pregnancy-associated plasma protein-A; STC, stanniocalcin; ELISA, enzyme-linked immunosorbent assay; CM, conditioned medium; IP, immunoprecipitation; WB, Western blot; hDPC, human dental pulp cells

* Corresponding author.
E-mail address: J.Beattie@leeds.ac.uk (J. Beattie).

https://doi.org/10.1016/j.ghir.2018.07.003
Received 8 June 2018; Received in revised form 16 July 2018; Accepted 19 July 2018
Available online 26 July 2018

may suggest a co-ordinated response of the IGF axis during osteogenic differentiation of hDPCs, differentiating osteoblasts also express IGFBP-4, -5 and -6 [11–13] and IGFBP-4 has been reported as the most abundant IGFBP in bone tissue [14]. To obtain a comprehensive account of IGF axis action in any cell culture system it is necessary to obtain full qualitative and quantitative data on IGF axis gene expression in order to be confident about how changes in gene expression and activity may impact on chosen biological endpoints. Surprisingly, with regard to quantitative aspects of IGF axis expression, very few studies have reported detailed profiles of gene and protein expression within cell culture models. In addition to this, IGFBPs are subject to post-translational modifications which have significant effects on their activity. Perhaps foremost amongst these is the proteolysis of IGFBPs into fragments with reduced IGF affinity. By this mechanism IGFBP proteolysis may regulate the partition of IGFs between high affinity IGFBPs and lower affinity cell surface receptors [15]. IGFBP proteases represent a broad range of enzyme activities with varying degrees of substrate specificity and it is important that the activity of these enzymes is acknowledged during investigations of IGF axis activity in selected cell and tissue culture models [16].

Dental tissues develop from oral ectoderm and neural crest derived mesenchyme and contain pluripotent stem cell populations which display a developmental potential similar to embryonic stem cells (ESCs) [17, 18]. Dental pulp tissue represents an accessible source of such cells and human dental pulp cells (hDPCs) have been extensively used in various cell differentiation studies [19–21]. Undifferentiated cells display a fibroblast-like morphology with associated high efficiency for adherent colony formation and high proliferative potential [22]. These properties suggest that adult dental tissues may provide a source of material (often discarded in the clinic) to provide multipotent cells for subsequent tissue engineering studies. To date the main use of such cells has been in hard tissue engineering programmes with a view to treatment of dental trauma. As such hDPCs have been differentiated down osteogenic/odontogenic lineages to generate appropriate 3-dimensional bone and or dentin structure [23, 24]. Although such strategies have met with some limited success they are hampered by an inadequate understanding of the effects of local growth factors on dental pulp cell differentiation. In the context of osteogenesis the IGF axis is known to play a crucial role in both the maintenance and differentiation of bone tissue (reviewed in [9] and see above). Similarly some recent studies have suggested a role for IGFs in regulating osteogenic/odontogenic differentiation of hDPCs [25–28] although the majority of these studies have given little attention given to the role of IGFBPs in this process. Therefore in the current study we have used the hDPC culture model to report comprehensively on IGF axis expression and activity during the osteogenic differentiation of these cells. In addition we investigate the activity of the specific IGFBP-4 proteinase, pregnancy-associated plasma protein-A (PAPP-A), which is expressed as the sole IGFBP-4 protease by hDPCs, and the potential regulation of PAPP-A activity by stanniocalcin-2 (STC2) -a recently described proteinase inhibitor of PAPP-A. We describe a series of co-ordinated changes in IGF axis expression which we suggest may represent part of an “osteogenic signature” associated with differentiating hDPCs.

2. Materials

Tissue culture medium α-MEM, foetal bovine serum (FBS) and phosphate buffered saline (PBS) were from Lonza (Slough, UK). Penicillin/streptomycin (PS), L-glutamine, L-ascorbic acid and dexamethasone, were from Sigma (Dorset, UK). Tissue culture plastic was from Corning (Amsterdam, Netherlands). Taqman probes and primers were from ABI (see Supplementary Table 1). ELISA kits for assay of IGFBP-2 (#DGB200), IGFBP-3 (#DGB300), IGFBP-4 (#DY804), IGFBP-5 (#DY875), IGFBP-6 (#DY876), IGF1 (#DG100) and IGF2 (#DG200) were from R&D Systems (Abingdon, UK). RhIGFBP-4 (#804-GB), rhIGFBP-5 (#875-B5), rhIGF-1(#291-G1) and rhIGF-2 (#292-G2) were also from R&D Systems. Reagents for STC2 ELISA including recombinant (r) STC2 are described in [29]. Recombinant human (rh) PAPP-A was expressed and purified as described in [30]. PAPP-A antibodies for IP are described in [31]. PAPP-A (pico) ELISA kit (Ansh, TX, AL-101) was used for ELISA. Polyclonal rabbit anti-PAPP-A antibodies for WB following IP are described in [32]. Reagents for electrophoresis including 8–16% gradient polyacrylamide gels, ×4 SDS-PAGE sample buffer and molecular weight markers were from BioRad.

3. Methods

3.1. Tissue culture

Isolation, culture and osteogenic differentiation of DPCs have been described previously [10, 33]. These cells represent a heterogeneous population of stromal cells which contain a subset of cells which express the classical cell surface marker profile (CD90+/CD105+/CD146+/CD45-/CD31-) characteristic of pluripotent mesenchymal stem cells [34–36]. Cells were generally used between passage 3–5. Serum free medium was collected over a 24 h period from basal or differentiated cells. Typically media were collected at the end of a 7 day or 21 day differentiation period. Further details are provided in relevant figure legends. After freeze drying medium was reconstituted at ×5 concentration, aliquoted and stored at −80 °C prior to analysis.

3.2. ELISA

ELISA of IGFBP-2-6, IGF1, IGF 2 and STC2 and PAPP-A were carried out using capture based ELISA kits sourced as described above. CM samples were assayed at dilutions which fell within the standard curve range appropriate for the analyte in question. Unconditioned medium was used as a zero analyte control and to examine potential background reactivity. At the dilutions used no non-specific binding was evident. All other details of ELISA were conducted exactly according to manufacturer's instructions.

3.3. Western blot (WB)

CM samples were analysed by reduced SDS-PAGE following electrophoresis through 8–16% gradient polyacrylamide gels. Following electrophoresis (1 h; 100 V) gels were blotted to PVDF membranes using the semidry TurboBlot™ apparatus. Blots were blocked for 1 h in 0.1% TBS-Tween (TBS-T) containing 5% (w/v) BSA. Blots were subsequently hybridised with primary antibody (1:1000 in TBS-T for 1 h at RT or overnight at 4 °C). After ×3 washes in TBS-T blots were incubated with HRP conjugated secondary antibody for 1 h at RT, then washed ×3 in TBS-T and developed with SuperSignal™ enhanced chemiluminescent (ECL) reagent. Images were visualized, captured and semi-quantified using the GelDoc imaging system (BioRad). Estimation of the Mr of reactive bands was also achieved using GelDoc software along with concurrently run Mr standards.

3.4. qRT-PCR

TaqMan based real time quantification of target mRNA abundance was performed using an LC480 light cycler (Roche) as described previously [37]. GAPDH was used as housekeeping gene (HKG). Target genes are quantified as $2^{-\Delta Ct}$ relative to GAPDH and fold changes in gene expression basal v osteogenic are expressed as $2^{-\Delta\Delta Ct}$. Supplementary Table1 lists TaqMan assay identifiers.

3.5. Bioassay

IGF1 stimulation of ALP activity was examined over the range 0–100 nM exactly as described in detail previously [10]. When present IGFBP-4 and IGFBP-5 were co-incubated at 10 nM along with IGF1.