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Leading opinion

Re-evaluating the induction of bone formation in primates

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ABSTRACT

The molecular cloning of the osteogenic proteins of the transforming growth factor-β (TGF-β) supergene family and the results of numerous pre-clinical studies in several mammalian species including non-human primates, have prematurely convinced molecular biologists, tissue engineers and skeletal re-constructionists alike to believe that single recombinant human bone morphogenetic/osteogenic proteins (hBMPs/OPs) would result in tissue induction when translated in clinical contexts. This theoretical potential has not been translated to acceptable clinical results. Clinical trials in craniofacial and orthopedic applications such as mandibular reconstruction and sinus-lift operations have indicated that supra physiological doses of a single recombinant human protein are needed to induce unacceptable tissue regeneration whilst incurring significant costs without achieving equivalence to autogenous bone grafts. The acid test for clinically relevant bone tissue engineering should now become the concept of *clinically significant osteoinduction*, whereby the regenerated bone is readily identifiable on radiographic examination by virtue of its opacity and trabecular architecture. The need for alternatives to the hBMPs/OPs is now felt more acutely following reported complications and performance failure associated with the clinical use of hBMP-2 and hOP-1 (BMP-7). Because of the often substandard regeneration of clinical defects implanted with hBMPs/OPs, we now need to finally deal with the provocative question: are the hBMPs/OPs the only initiators of the induction of bone formation in pre-clinical and clinical contexts? The rapid induction of bone formation by the hTGF-β_3 isoform in heteropic intramuscular sites of the Chacma baboon *Papio ursinus* together with *TGF-β_1*, *TGF-β_3*, *BMP-2*, *BMP-3*, *OP-1*, *RUNX-2* and *Osteocalcin* up-regulation and expression, hyper cellular osteoblastic activity, osteoid synthesis, angiogenesis and capillary sprouting are the molecular and morphological foundation for the induction of bone formation in clinical contexts. The induction of bone as initiated by hTGF-β_3 when implanted in the *rectus abdominis* muscle of *P. ursinus* is *via* the *BMPs/OPs* pathway with hTGF-β_3 controlling the induction of bone formation by regulating the expression of *BMPs/OPs via Noggin* expression, eliciting the induction of bone formation by up-regulating endogenous BMPs/OPs and it is blocked by hNoggin, providing insights into performance failure of hBMPs/OPs in clinical contexts. Physiological expression of *BMPs/OPs* genes upon implantation of hTGF-β_3 may escape the antagonist expression of Noggin and other inhibitors, whereas direct application of hBMPs/OPs, representing a later by-product step of the bone induction cascade as set by the TGF-β_3 master gene in primates, sets into motion Noggin' antagonist action, as shown by the limited effectiveness of hBMPs/OPs in clinical contexts. The unprecedented induction of bone formation by 250 μg hTGF-β_3 when combined with coral-derived macroporous constructs is the novel molecular and morphological frontier for the induction of bone formation in man. The induction of bone by hTGF-β_3 has been thus translated in clinical contexts to treat a large mandibular defect in a pediatric patient; 30 months after implantation of 250 μg hTGF-β_3 per gram of human demineralized bone matrix, radiographic analyses show the reconstruction of the avulsed large mandibular segment including the induction of the avulsed coronoid process.

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1. Introduction: regenerative medicine and bone: formation by autoinduction

Scientists should often critically review not only the results achieved in specific research endeavors but particularly critically appraise the scientific performance of newly developed disciplines with a view to focus, sharpen, improve and perhaps even amputate poorly performing research avenues when translated in clinical contexts.

Regenerative medicine is a rapidly expanding field with major developmental, biological, molecular and surgical advances that have clinically outrun the realities of bone tissue engineering.

The remarkable successes in pre-clinical animal models have helped to create the continuous building blocks of tissue engineering and regenerative medicine at large, tempting and suggesting molecular biologists and surgeons alike that the era of tissue reconstruction of spare parts of the human body is finally close at hand.

Several reviews and perspectives have highlighted an inexorable series of research discoveries paving the way to translational research in clinical contexts [1–4]. The bulk of such reviews and future perspectives in tissue engineering conclude a bright future for regenerative medicine highlighting how tissue engineered technologies have been developed for therapies in clinical contexts hypothesizing further important developments for human patients [1,4].

Indeed, it is stated that major "advances are now beginning to change the lives of small subsets of the population" [1]. This hype of potential therapeutic translation in clinical contexts has been further fueled by the recent Nobel prize awarded for induced pluripotent stem cells [5,6] generating induced embryonic stem cells for regenerative therapies [7].

It is our opinion however, supported by few others [8] that in spite of the tremendous advances in cellular, molecular, developmental and surgical biology, the as yet unmet acid test of regenerative medicine and tissue engineering is the predictable, successful translation in clinical contexts of the novel therapeutic strategies so brilliantly developed in pre-clinical animal models.

We are in full agreement with Martin's statement [Martin 2014] that merely hypothesized, yet published advanced tissue engineering perspectives, have been published "*even in the awareness that the need of such functionalities is largely not substantiated by experimental data*" [9]. The *conundrum* of regenerative medicine and tissue engineering had been a newly developed research program [10,11] which later morphed into the hyperbole of promised novel regenerative treatments based on published data in pre-clinical animal models [12] without any experimental evidence of translational research in clinical contexts. Reviews and perspectives on bone tissue engineering report a series of successful novel procedures in animal models with the promise that the results obtained both *in vitro* and *in vivo* will eventually result in substantial differences in acute and chronic human disorders including but not limited to, myocardial infarction following transplantation of functional contractile myoblastic cells, liver, pancreas and kidney failure following transplantation of bioactive hepatocytes, healthy grown pancreatic islets as well as supra-assembling kidney tubular structures with filtering cells [12]. As reported by Williams [8], none of the above tissue engineering procedures are actually routinely used in clinical contexts.

Levander's "*Tissue induction*" paper published in Nature sets the rule of tissue engineering, that is "regeneration of tissues is a repetition of embryonic development" [13].

There is a direct relationship between differentiation processes in embryonic development and postnatal tissue regeneration [13–19]. The osteogenic proteins of the TGF-β superfamily are the common molecular initiators deployed for embryonic development and postnatal tissue induction and regeneration, whereby soluble molecular signals, deployed in embryonic development, are re-deployed post-natally to induce tissue induction and morphogenesis as a recapitulation of embryonic development [19–21].

Recapitulating embryonic development [13–22] thus merges with the modern concept of "developmental engineering" [23]. In previous experiments in adult non-human primates we have established the primary role of naturally-derived highly purified bone morphogenetic/osteogenic proteins (BMPs/OPs) in the induction of osteogenesis [16,24,26], and stated that the capacity of mammalian naturally-derived osteogenic proteins to initiate a programmed cellular cascade that results in the induction of bone formation is a functionally conserved process utilized in embryonic development, recapitulated in post fetal osteogenesis, and can be re-exploited for the therapeutic induction of bone formation [16], thus predating the more recent concepts of "developmental engineering" [23] and of "re-engineering development" [24].

2. Translational regenerative medicine: the induction of bone formation

Regeneration of adult tissues is largely the deployment of stem cell functions recapitulating embryonic development and morphogenesis [14–16,19,20,22]. Beyond multiple stem cell niches in organs and tissues, however, there is the clinical bed side [20] and the hype of stem cell science now needs to be translated in clinical contexts [27–30] to finally prove its speculative functionalities.

Above all, however, the future of regenerative medicine is not only to use stem cells but rather to construct biomimetic biomaterial matrices that *per se* and without the exogenous application of morphogenetic signals or morphogens, first described by Turing as "forms generating substances" [31], initiate the multistep cascade of gene expression and secretion and the induction of tissue formation or morphogenesis [32–35].

Biologically, translational regenerative medicine is comparable to the induction of bone formation [Urist 1965; Reddi and Huggins1972; Reddi 1981]. The acid test for the induction of bone formation is *de novo* generation of heterotopic bone after extraskeletal implantation of an osteogenic device deemed to be osteoinductive [20,36–39].

A protein and/or a biomimetic self-inductive matrix must thus be endowed with the striking prerogative of initiating the induction of bone formation in heterotopic extraskeletal sites of animal models [20,34,39,40] (Fig. 1). This is a mandatory prerequisite to define an osteoinductive soluble molecular signal [36,37] or a self-inductive biomimetic matrix [33]. The heterotopic implantation site avoids the ambiguities of the orthotopic site where bone formation by conduction may occur from the viable bone interfaces [34,36,38], particularly when using *smart* biomimetic matrices as bone repair materials [34,41].

Similarly, translational medicine must be routinely deployed in humans to define a successful tissue engineering procedure based on predictable results obtained in animal models including non-human primates [40–45]. In context, BMPs/OPs have failed to translate exceptional results in pre-clinical contexts including non-human primate models [34,40–45].

Did bone tissue engineering and BMPs/OPs research provide opportunities to gain insights into the inhibitory mechanisms regulating the induction of bone formation in human clinical trials? To the best of our knowledge, only few studies provided some insights into limited biological activity or the need of mega doses of the recombinant proteins [46].

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