FEATURES

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3D Co-Culture Facilitates Differentiation of ESCs Into Cardiomyocytes

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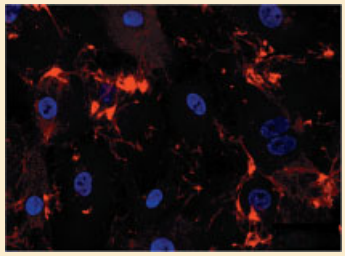

Cardiomyocyte (CM) differentiation of embryonic stem cells (ESCs) is routinely cultured as a two-dimensional (2D) monolayer, which does not mimic the *in vivo* physiological environment and may lead to low differentiated level of ESCs. Here, Ou et al develop a novel strategy that enhances CM differentiation of ESCs in collagen matrix three-dimensional (3D) culture combined with indirect cardiac fibroblast co-culture. ESCs were cultured in hanging drops to form embryoid bodies (EBs) and then applied on a collagen matrix. The EBs were indirectly co-cultured with cardiac fibroblasts by the hanging cell culture inserts (PET 1μm). Molecular expressions and ultrastructural characteristics of ESC-derived CMs (ESCMs) were analyzed by real time RT-PCR, immunocytochemistry and TEM. The authors found that the percentage of beating EBs with cardiac fibroblast co-culture was significantly higher than that without co-culture after a differentiation period of 8 days. Type I collagen used as 3D substrates enhanced the late-stage CM differentiation of ESCs and had an effect on the ultrastructural maturity of ESCMs in late-stage development. The combined effects of 3D and co-culture that mimic the *in vivo* physiological environment further improved the efficiency of CM differentiation from ESCs, resulting in fiber-like structures of cardiac cells with organized sarcomeric structure in ESCMs. This novel 3D co-culture system emphasizes the fact that ESC differentiation actively responds to cues from the environment. These cues can drive phenotypic control, and provide a useful *in vitro* model to investigate CM differentiation of ESCs.

Runx2-HIF-1α Induces VEGF

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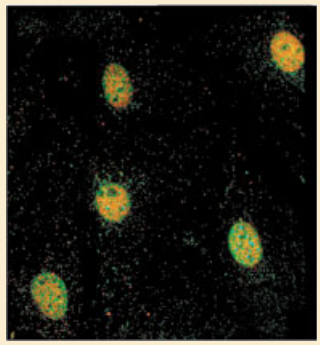

The intimately related processes of angiogenesis and bone formation are largely controlled by two important transcription factors, HIF1α and RUNX2. Genetic studies established essential roles for both factors in osteogenesis. Although HIF-1α and RUNX2 are thought to independently control osteogenesis, respectively, by providing a vasculature and functional osteoblasts, cross-talk between these two factors is also observed; RUNX2 can induce the angiogenic peptide, VEGF, while HIF-1α affects osteoblast activity. In this study, Kwon and coworkers provide direct evidence for RUNX2-HIF-1α interactions by showing that these two factors physically and functionally interact in osteoblasts to increase VEGF expression. Although RUNX2 is not essential for HIF-1α-dependent induction of VEGF in hypoxia, it synergistically stimulates this response. Similarly, inhibition of HIF-1α totally blocks VEGF induction by RUNX2. These two factors were co-localized to specific nuclear sites and detected on common regions of VEGF chromatin. Finally, co-immunoprecipitation studies showed that HIF-1α directly binds to RUNX2 via a specific site in the runt domain. The work presented shows that RUNX2 and HIF-1α cooperatively interact in osteoblasts to stimulate an angiogenic gene, thereby providing a direct link between vascularization and osteogenesis. This interaction may also be relevant to understanding regulation of osteogenic precursor cells that are components of the vasculature.

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Persistent Infectivity after Prion Protein Destruction

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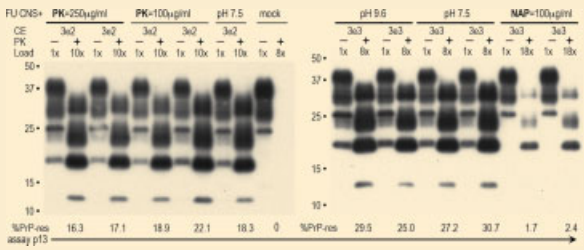

The hypothesis that host prion protein (PrP) converts into an infectious prion form rests on the observation that infectivity decreases in direct proportion to the decrease of proteinase K (PK) resistant PrP (PrP-res, PrP^{sc}). This infectious prion form somehow encodes the many unique TSE agent-strains. Recently, a PK sensitive form of PrP has been proposed as the prion. Miyazawa et al re-evaluated the relationship of total PrP (sensitive and resistant) to infectious titers. A keratinase (NAP) known to effectively digest PrP was compared to PK. Total PrP in FU-CJD infected brain decreased to $\leq$0.3% in a 2hr PK digest, yet there was no reduction in titer. Remaining non-PrP proteins were easily detected in this highly infectious homogenate. In contrast to PK, NAP digestion left 0.8% residual PrP at 2hrs, yet decreased titer by >2.5logs; few protein bands remained. FU-CJD cultures with 10x the infectivity of brain also showed that NAP significantly reduced infectivity (>3.5 logs). Extreme PK digestions were required to reduce cell PrP to <0.2%, yet a very high titer of $\geq$7.8 logs survived. It is therefore unlikely that any form of PrP is infectious (a prion). One or more residual non-PrP proteins probably protects a strain-specific nucleic acid.

HIF-1α and The Regulation of Tumor Angiogenesis

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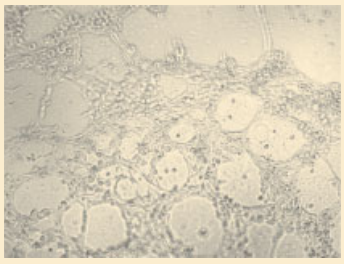

Tumors are dependent on angiogenesis for adequate nutrient and oxygen delivery as well as for metastatic dissemination. Hypoxia-inducible factor 1-α (HIF-1α) is a transcription factor that initiates transcription of genes necessary for hypoxic adaptations including angiogenesis. In normoxia, HIF-1α is regulated through hydroxyl-dependent interactions with the E3 ubiquitin ligase, von Hippel-Lindau (VHL). However, regulation of HIF-1α during hypoxia is poorly understood. Using an ovarian cancer cell model, Cassavaugh et al. identified a novel mechanism for hypoxic regulation of HIF-1α that is mediated by glycogen synthase kinase 3β and the E3 ubiquitin ligase, FBW7. Suppression of GSK3β or FBW7 increased HIF-1α stability and reduced its ubiquitination during hypoxia. FBW7 was also shown to interact with HIF-1α in hypoxia and this interaction was significantly reduced with GSK3β inhibition or mutation of consensus GSK3β phosphorylation sites on HIF-1α. Furthermore, conditioned media from cells with suppressed GSK3β or FBW7 in hypoxia significantly increased endothelial cell tube formation, suggesting a role for this regulatory mechanism in angiogenesis. In light of the fact that many metastatic tumors are hypoxic, a novel mechanism to regulate HIF-1α during hypoxia is particularly relevant towards understanding the regulation of tumor angiogenesis.