Diabetic glucose impairs embryonic stem cell osteogenic differentiation through persistent activation of Akt

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Abstract

Objectives:
The mammalian embryo is said to have a high amount of plasticity, as observed by the ability of the embryo to develop in a wide variety of conditions in vitro. Following fertilization, many changes occur to an early stage embryo in regards to metabolism and embryonic nutrition. The requirement for glucose (Glc) during development is thought to be for the biosynthesis of macromolecules such as phospholipids and nucleic acids, however studies have shown that diabetic Glc levels can be toxic to the developing embryo. For instance, newborns of diabetic mothers may exhibit skeletal malformations. Embryonic stem cells (ESCs) are pluripotent cells that are capable of modeling osteogenesis and thus often serve researchers to study embryonic skeletal development. Against this background, we aimed to gain insight into the molecular mechanism whereby Glc may influence the process of osteogenesis.

Methods:
Murine ESCs were differentiated in media containing either physiological (1.0 g/l) or diabetic (4.5 g/l) concentrations of D-glucose while simultaneously being triggered into osteogenesis using 1alpha,25(OH)2 vitamin D3. The osteogenic differentiation ability of the cells was evaluated by histochemical stainings, quantitative bone marker gene expression and flow cytometric analyses at distinct time points of maturity. In addition, the calcification of the secreted extracellular matrix was quantified and signaling pathway activation studied.

Results:
mRNA expression and van Gieson staining suggested that more collagen was produced and secreted by cells differentiated in diabetic conditions, while matrix calcification was severely impaired, suggesting that cells were prevented from maturation. Instead, cells differentiated in physiological Glc conditions showed normal osteogenic progression, including stage specific activity of alkaline phosphatase. Follow-up studies then revealed that the Glc-mediated osteogenic impairment was already detectable at the level of an osteoprogenitor state. Western blotting then allowed us to correlate persisting Col I mRNA expression to elevated protein levels of AKT and activated AKT (phosphor-S473), which has been previously reported to control Col I mRNA expression in other cell types. Stage-specific blockage of AKT activation using the AKT inhibitor 124005 restored normal Col I mRNA levels and led to a calcification rescue in diabetic conditions.

Conclusions:
In conclusion, we were able to show that diabetic Glc inhibits the formation of matrix calcifying osteoblasts in embryonic stem cell cultures possibly through AKT mediated persistent expression of Col I mRNA.