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E2F6 attenuates the hypoxia mimetic cobalt chloride induced apoptosis of cardiomyocytes.

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Abstract

Introduction: The E2F pathway governs cell growth, differentiation, and death in all cell types. To interrogate the role of this pathway in postnatal myocardium we generated a transgenic mouse model (Tg) with cardiac specific expression of the repressor E2F6 which deregulates the E2F response (1). The Tg mice developed a E2F6 dose dependent dilated cardiomyopathy in the absence of any hypertrophy or cell death (1,2). Here we examined whether E2F6 serves as an anti-apoptotic agent to attenuate cell death of postnatal cardiomyocytes to drugs such as doxorubicin (dox) and the hypoxia mimetic agent cobalt chloride ($CoCl_2$).

Methods/Results: Microarray analysis of wild type (Wt) E2F6- transgenic (Tg) mice revealed an increase in genes which regulate the DNA damage response which was confirmed by RT-qPCR: Chek1 kinase (3.6 fold, p-value: 0.0005), Rad51 (2 fold, p-value: 0.01), and Blm2 (4 fold, p-value: 0.006). Western blot analysis of Chek1 confirmed a threefold up-regulation at the protein level (p-value: 0.022). Neonatal cardiomyocytes (NCM) were isolated from Wt and Tg mouse myocardium at post-natal day 1 and treated with dox or $CoCl_2$. At 500nM dox induced caspase 3 cleavage and p53 acetylation in both Wt and Tg NCM to a similar extent. E2F6 expression was down-regulated following 3 hours of dox exposure and completely abolished after 24hr. In HeLa cells, dox also targeted the endogenous degradation of E2F6 (50% reduction, p-value: 0.04; with 500nM dox; 90% reduction, p-value: 0.008 with 1uM), while the apoptotic E2F1 was up-regulated (1.4 fold p:0.04; 500nM dox and 2 fold,p-value:0.004 by 1uM). Treatment of NCM with the $CoCl_2$ revealed that E2F6 attenuated the induction of caspase 3 cleavage by 50 fold (p-value:0.02) as well as the diminishing the bax: bcl2 ratio. $CoCl_2$ treatment of Wt NCM resulted in a two fold increase in the number of TUNEL positive cells while E2F6 Tg NCM were unaffected. Unlike dox, $CoCl_2$ treatment did not inhibit E2F6 levels in NCM further demonstrating that E2F6 is anti-apoptotic.

***Conclusion*:** The data demonstrate that E2F6 protects against the drug induced apoptosis of cardiomyocytes. Further, dox appears to confer cell death via post-transcriptional mechanisms targeting the degradation of E2F6 and upregulation of the proapoptotic E2F1.

References

1.	Westendorp B, et al. (2012). *FASEB J.* **26**: 2569-2579.
2.	Major JL et al. (2015). *J Mol Cell Cardiol*. **84**: 179-190.