Megakaryocytic Gene Regulation in Response to Shear Stress

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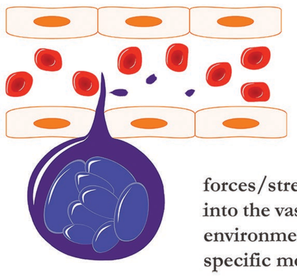
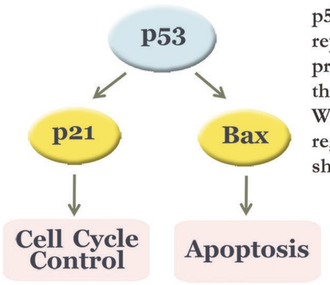
Introduction: Megakaryocytes Are Subject to Shear Stresses

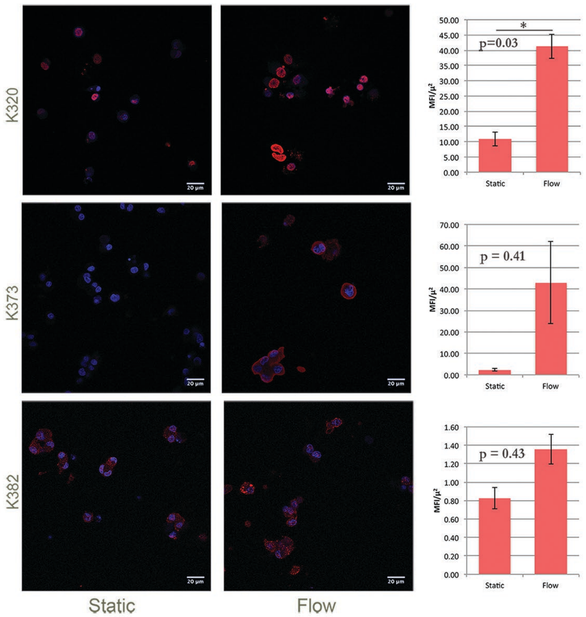


Megakaryocytes (Mks) are large, polyploid cells that reside in the bone marrow and differentiate from the CD34+ hematopoietic stem cell (found within the stem-cell compartment of the marrow) and are known for producing platelets, the cell fragments responsible for blood clotting. A more thorough understanding of how Mks produce platelets is important for developing better therapies and producing platelets in vitro for blood transfusions. Many factors play a role in Mk differentiation, one being shear forces/stresses. MKs are subject to shear stresses as they travel from the bone marrow into the vasculature. The responses to these stresses are investigated using an engineered environment with systems biology approaches. Our data indicate that shear stresses elicit specific molecular response that may help to complete maturation and produce platelets.

p53 Acetylation at K320, K373, & K382 Increases in Response to Shear Stresses



p53 is a protein often described as the "guardian of the genome". It fulfills this role by inducing cell cycle arrest and DNA repair when necessary. If genotoxic damage (i.e. UV irradiation) becomes too extensive, p53 will direct the cell toward programmed cell death (apoptosis). These processes are implicated in Mk maturation. Previously in our lab, we demonstrated that p53 knock out (KO) increases the percentage of Mks in a culture, increases DNA synthesis, and decreases apoptosis [1]. While the number of platelets in p53 KO mice is normal, they are significantly less functional. Thus, p53 could be a central regulator of both Mk maturation and platelet production. p53 activity is highly reliant upon acetylation patterns, which is shown to regulate gene promoter binding.

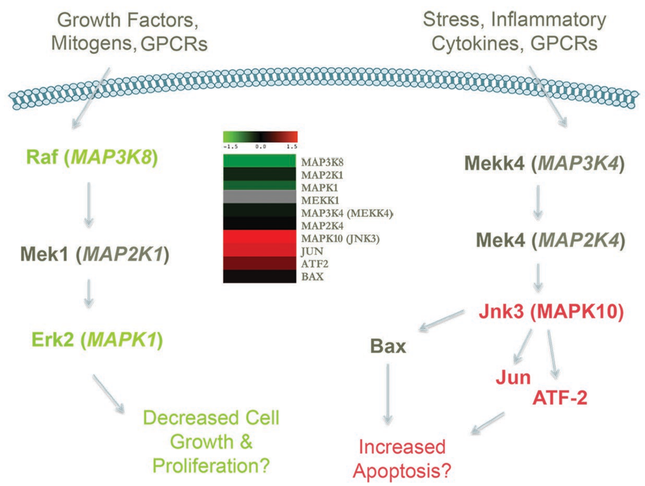


In endothelial cells, changes in p53 acetylation patterns have previously been associated with shear stress [2]. Here, the data indicate that in Mks, p53 is acetylated differently than it is in endothelial cells. Acetylation at three residues (K320, K373, and K382) increases in response to shear stresses. Interestingly, this pattern resembles that of genotoxic stress, such as UV irradiation. This acetylation pattern may have downstream implications in transcriptional regulation.

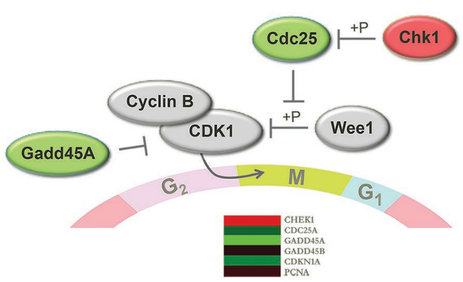
Blue = DAPI; Red = acetyl-p53
Three biological replicates with CD41+ Mk primary cells; static cells received no shear stress; flow cells had shear stress at 1.0 dyne/cm² for 2 hours.

Gene Expression Microarray Analysis Indicates that Shear Stresses Result in Increased Expression of Genes Related to Cell Cycle Arrest, Apoptosis, & Microtubule Sliding

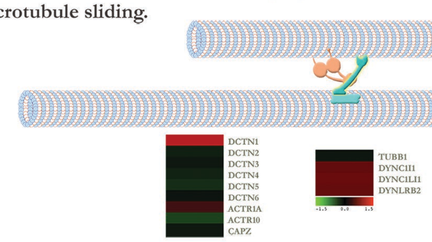
There is a complex interrelationship between the apoptotic process of terminally differentiated megakaryocytes and platelet release. Our data indicate that shear stresses result in downregulation of genes involved in growth and proliferation, while genes involved in a particular apoptotic pathway are upregulated. Jnk, which regulates Jun-mediated apoptosis and Bax activation for intrinsic apoptosis, are upregulated in response to shear stresses. Thus, it is likely that shear stresses accelerate apoptosis and decrease proliferation to encourage platelet production.



Endomitosis is a modified form of the cell cycle with a characteristic failed cytoplasmic division (cytokenesis) that produces polyploid cells (8N+) with multilobulated nuclei [4]. Physiologically, shear stresses could contribute to cell cycle arrest so that cellular resources can be directed towards platelet production. Our data indicate that cell cycle arrest occurs simultaneously with increased apoptosis. Checkpoint kinase 1 (Chk1), a key player in cell cycle arrest, is upregulated in response to shear stresses, which could potentially be a mechanism for terminating endomitosis. GADD45A, which is responsible for DNA repair during cell cycle arrest, is downregulated. This may act to encourage apoptosis, rather than performing DNA repair, during the arrest.



The sliding of microtubules, a cytoskeleton component, was shown to be vital for the elongation of proplatelets (the cellular processes that extend outward from the cell body and the location of platelet release) [3]. DCTN1 (p150/ Glued), the main functional subunit of the Dynactin complex, is the largest driver of microtubule sliding. DCTN1 is upregulated in response to shear stresses, as well as other components of Dynein. This indicates that this shear stresses may accelerate the extension of proplatelets through increased microtubule sliding.



Unbiased Investigation of Microarray Analysis Reveals Metallotionein Downregulation

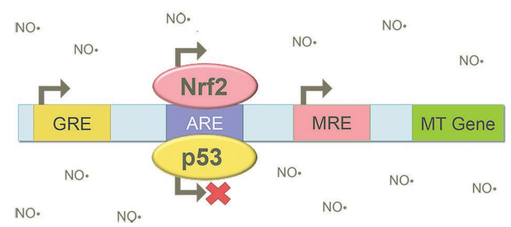
Most Downregulated Genes

	TF's	
MT1H: chr16	HEN1	*KLB*: chr4
MT1F: chr16	Sp1	*MT1E*: chr16
MT1A: chr16	TBP	*PAPOLB*: chr7
MT1L: chr16	USF1	*AGXT*: chr2
MT1JP: chr16	USF2	*MXI1*: chr10
	C-Myb	
	Meis-1	

Significance Analysis of Microarrays (SAM)

MT1G: -1.25	*MT2A*: -1.46
SECTM1: -1.61	*MT1B*: -1.66
MT1X: -1.74	*MT1E*: -2.64
MT1JP: -3.51	*MT1L*: -3.74
MT1A: -4.72	*MT1F*: -4.74
MT1H: -4.90	

Investigating the most differentially expressed genes does not reveal a predicted transcription factor (TF) that can account for the regulation of all the genes. They are also spread amongst many different chromosomes. The only trend is with the MT1 genes, which code for Metallothioneins and show over a 7-fold decrease in expression. Induction of these genes is linked to numerous stresses, including oxidative stress (through the antioxidant response element - ARE), heavy metal accumulation (metal response element - MRE), and glucocorticoid presence (glucocorticoid response element - GRE). Various TFs are responsible for regulating these promoters. The TF Nrf2 is most recognized for regulating the ARE in response to nitric oxide, a product physiologically relevant because it is generated by the endothelial cells that line the blood vessels. While repression through these promoters hasn't been studied as thoroughly, p53, however, is linked to transcriptional repression through the ARE [5]. Shear-activated p53 may act as a transcriptional repressor of the MT genes to decrease cell recovery and increase apoptosis.



Conclusions: Shear Stresses Promote Mk Maturation

Our data indicate that shear stresses promotes Mk maturation by:
(1) Transcriptionally encouraging apoptosis and microtubule sliding;
(2) Transcriptionally inhibiting cell growth and proliferation;
(3) Increasing p53 acetylation in a pattern that mimics genotoxic stress.
Also:
(4) Linking metallothionein gene expression to shear stresses is novel;

Acknowledgements & References

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