

[4/18/2008(Fri) 15:35~16:10/1<sup>st</sup> FL]

## **Lysyl-tRNA Synthetase Inhibits Various Shear Stress-stimulated Signaling Pathways in Endothelial Cells**

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Hemodynamic shear stress, the dragging force generated by blood flow, is known as an anti-atherogenic factor. We tested whether lysyl-tRNA synthetase (KRS) will be utilized as an agent controlling shear-sensing systems. KRS was previously known to be secreted as a pro-inflammatory agent. Here we found that KRS inhibited various shear-stimulated signaling pathways. We further found that KRS binds to detergent-resistant membrane (DRM), indicating that KRS binding molecules exist in DRM, specialized regions of the plasma membrane. DRM plays important roles in a variety of cellular processes and consists of gangliosides, signaling molecules and cytoskeletons. We then determined that KRS was colocalized with integrins  $\alpha 4$ ,  $\alpha 5$  and  $\alpha v\beta 3$ . In addition, KRS was shown to be associated with sialic acid, existing at the end of gangliosides. Interestingly, the adherent effect of KRS was inhibited by pretreatment with sialic acid. Moreover, treatment of endothelial cells with neuraminidase appeared to inhibit both the KRS adhesion to endothelial cells and shear-stimulated signaling. In conclusion, KRS is likely to be utilized as a vascular regulator.

**Lysyl-tRNA synthetase inhibits various  
shear stress-stimulated signaling  
pathways in endothelial cells**

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Atherosclerosis



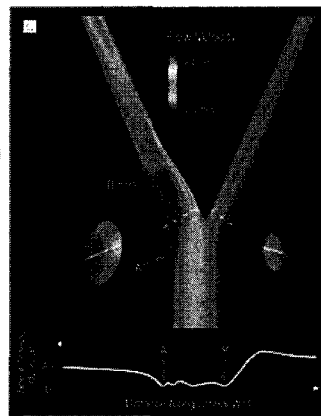
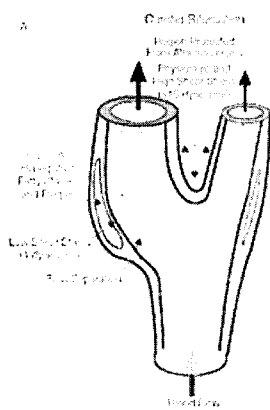
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**Shear stress: dragging force (frictional force) generated by blood flow**



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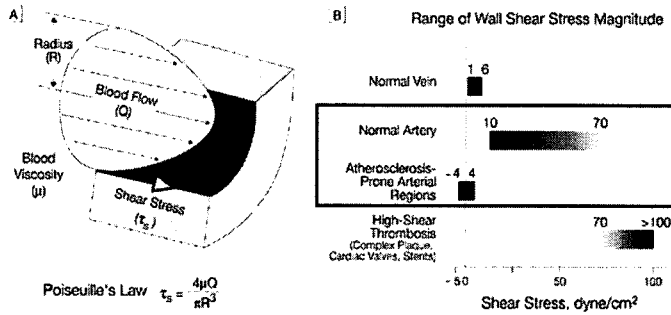
( JAMA 1999; 282:2035 - 2042 )



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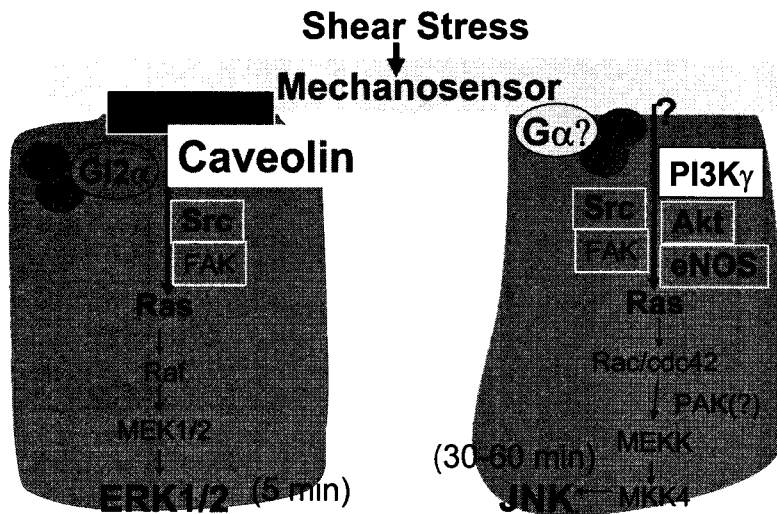
# Shear stress: an anti-atherogenic factor

( JAMA 1999; 282:2035 - 2042 )



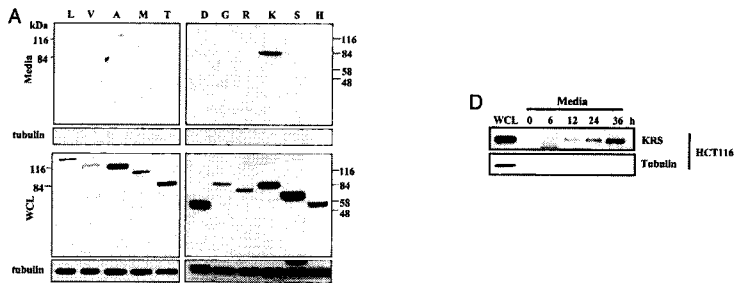
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## Temporo-spatial Segregation of Shear Signaling Pathways in BAECs



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**Human lysyl-tRNA synthetase is secreted to trigger proinflammatory response** (*PNAS* 102:6356-6361 (2005))

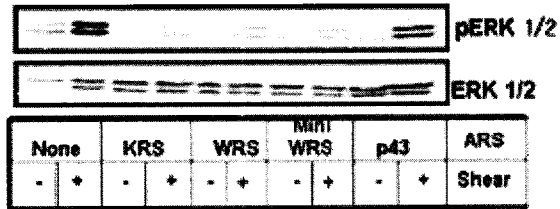


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**Does KRS play a role  
in shear stress-stimulated signaling?**

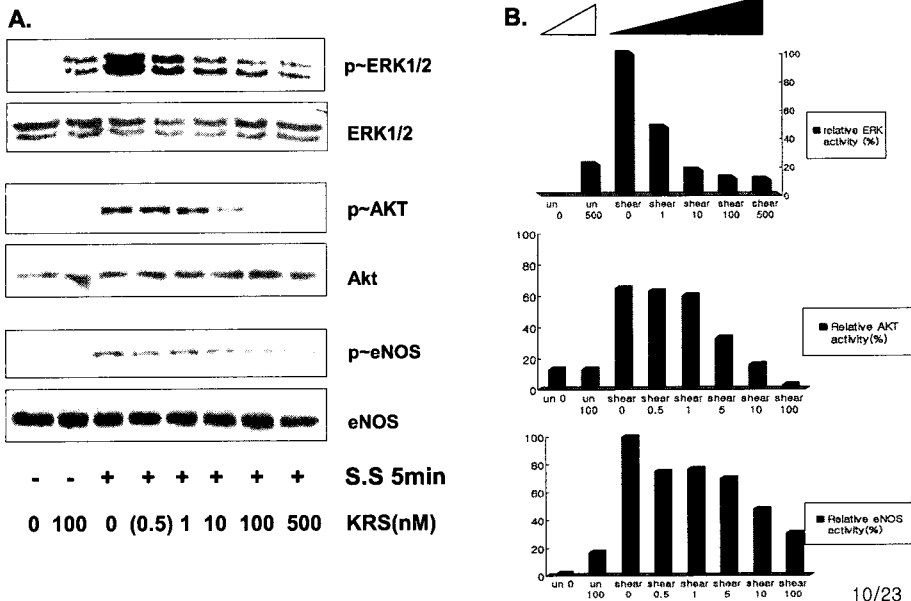
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## KRS (LysRS) inhibits shear-dependent ERK activity



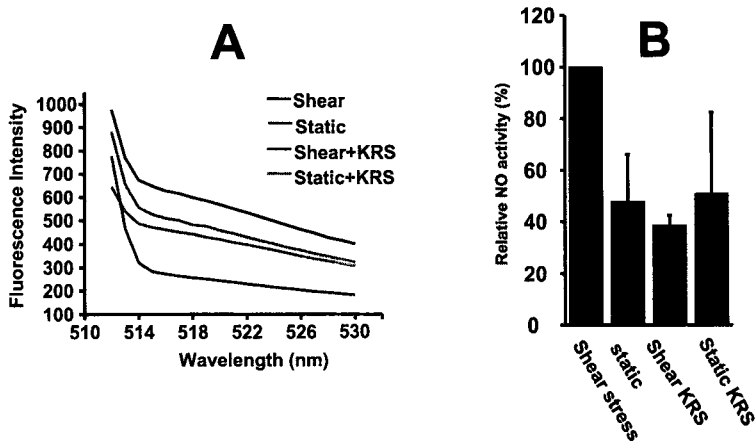
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## Shear-induced signalings were globally inhibited by KRS



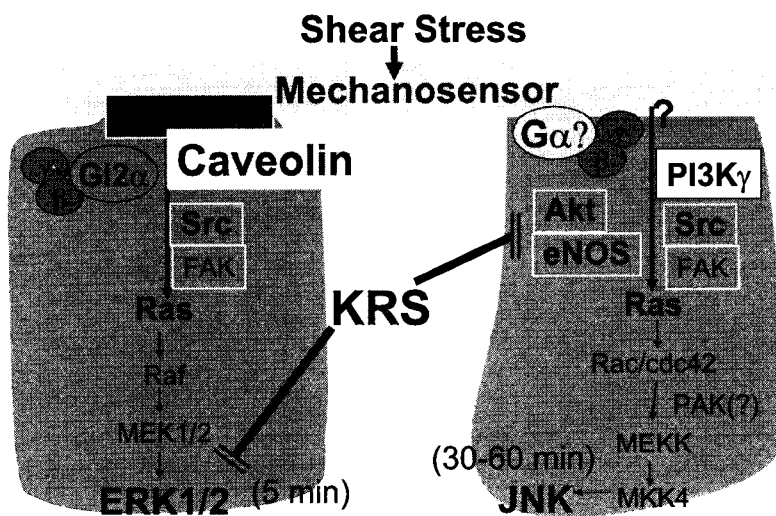
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**KRS blockades the shear stress-stimulated NO production**



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**KRS blocks shear-stimulated activation of ERK and JNK in BAECs**



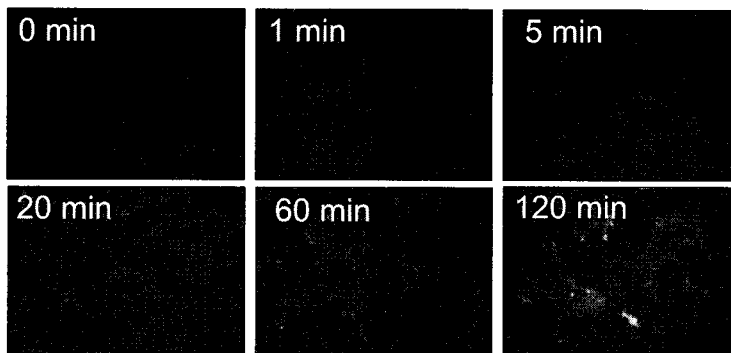
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## What is (are) shear sensing receptor(s)?

1. Integrins
2. Ion Channels
3. G-protein linked receptors
4. Caveolae
5. Glycocalyx

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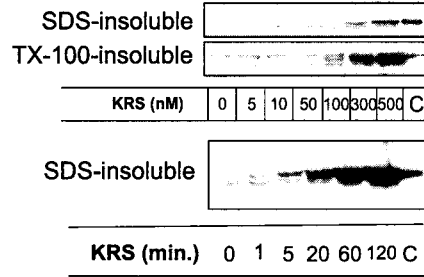
## KRS (500 nM) binds to the endothelial cell surface



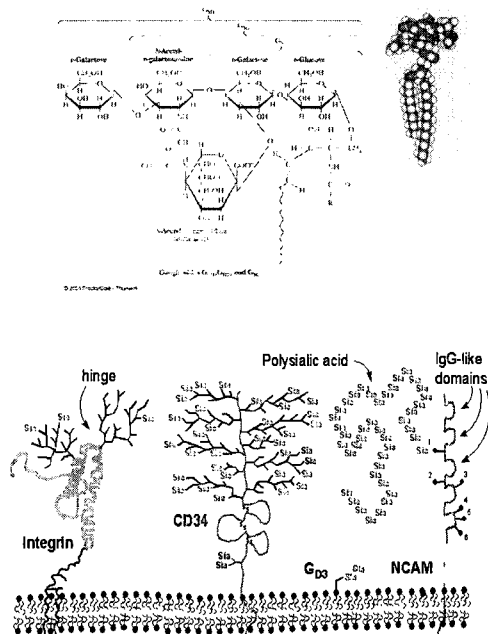
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## KRS binds to DRM



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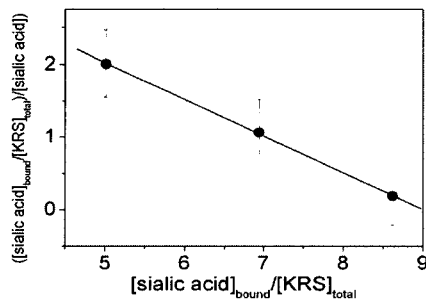
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Does KRS bind to sialic acid, a component of DRM?

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**In vitro binding assay for KRS to sialic acid**

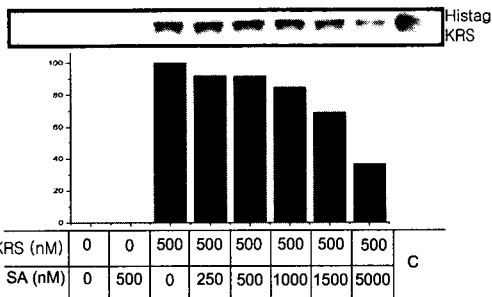
$K_d = 0.505 \pm 0.009 \mu\text{M}$



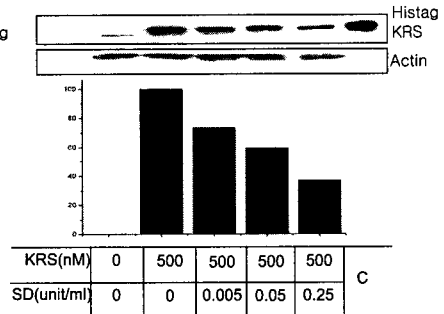
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## Sialic acid is an important factor for cell-binding of KRS

### A. Sialic acid competition

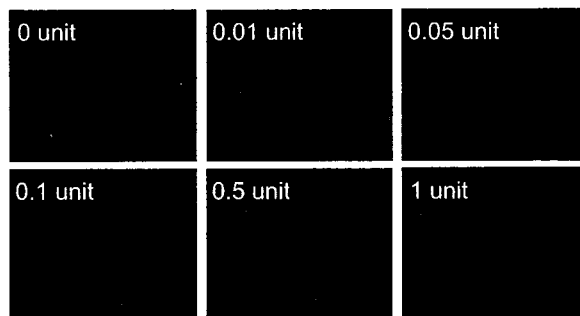


### B. Sialidase pretreatment



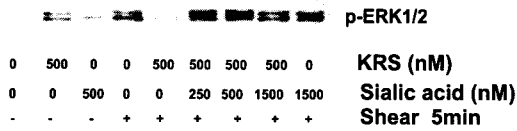
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### C. Sialidase pretreatment

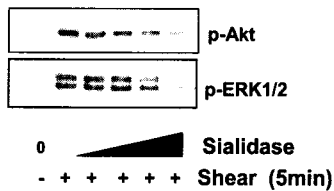


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**Sialic acid blocks the inhibitory activity of KRS**



**Sialidase inhibits shear-stimulated ERK activity**



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## Conclusions

- KRS binds to sialic acid in endothelial DRM.
- KRS inhibits shear-dependent signaling through the direct interaction with sialic acid.
- Sialic acid (glycocalyx) is an important component for shear-sensing

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