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MECHANOTRANSDUCTION

A stiff response

The stiffness of the extracellular environment can have profound effects on cellular properties and behaviours, such as intracellular stiffness (a correlate of intracellular tension) and proliferation. The mechanotransduction pathway that converts the sensing of extracellular matrix (ECM) stiffness by integrin-dependent focal adhesions into cellular changes is now described to involve a FAK–CAS–RAC1 (focal adhesion kinase 1–p130CAS (also known as CAS1 and BCAR1)–RAC1) stiffness-dependent signalling module.

The authors examined the phosphorylation status of focal adhesion proteins in serum-stimulated mouse embryonic fibroblasts (MEFs) plated on hydrogels of physiologically relevant low and high levels of stiffness. They showed that high-stiffness hydrogels induced phosphorylation of the adaptor protein CAS in MEFs. siRNA-mediated knockdown of *Cas* expression decreased entry into the S phase of the cell cycle of MEFs cultured on the high-stiffness substrate without disrupting the overall structure of focal adhesions, which indicates that CAS selectively transduces ECM stiffness into cell cycling.

Several lines of evidence in this study showed a role for FAK and SRC in the stiffness-sensitive phosphorylation of CAS. These findings led the researchers to develop a model in which high-stiffness-induced autophosphorylation of FAK increases the association of FAK with activated SRC (the phosphorylation of which occurs in a stiffness-independent manner). In turn, this increases SRC-mediated phosphorylation of FAK and hence increases phosphorylation of CAS by FAK and/or SRC. The phosphorylation of CAS in MEFs plated on high-stiffness substrates was decreased by FAK inhibition or SRC deficiency. Inhibiting the activity or expression of CAS, FAK or SRC decreased the abundance of the cell cycle protein cyclin D1 in MEFs plated on high-stiffness substrates, and decreased their entry into S phase.

In addition to the cell cycle effects, FAK and CAS were required to transduce extracellular stiffness into intracellular stiffness; FAK inhibition or CAS deletion decreased the intracellular stiffness of MEFs plated on high-stiffness hydrogels. In keeping with the fact that intracellular stiffness is determined by RHO-family-GTPase-mediated actin polymerization and that RAC1 is a known target of CAS, both RAC1 inhibition and CAS deletion had similar inhibitory effects on intracellular stiffness.

RAC1 also influenced the cell cycle, as shown by the increased cyclin D1 abundance in MEFs with constitutively active RAC1 plated on the low-stiffness substrate. These effects were shown to depend not only on the direct induction of cyclin D1 expression by RAC1 signalling but also on a positive feedback loop through which RAC1-dependent intracellular stiffening maintains FAK–CAS signalling.

Together, these data indicate that FAK–CAS–RAC1 signalling regulates both intracellular stiffness and cell cycling in response to extracellular stiffness. The physiological relevance of this pathway was shown in a mouse model of vascular injury that involves stiffening of the ECM surrounding vascular smooth muscle cells (VSMCs). VSMC-specific deletion of *Rac1* in this model resulted in decreased CAS phosphorylation, decreased cyclin D1 protein expression and decreased cell proliferation.

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ORIGINAL RESEARCH PAPER Bae, Y. H. *et al.* A FAK–Cas–Rac–lamellipodin signaling module transduces extracellular matrix stiffness into mechanosensitive cell cycling. *Sci. Signal.* **7**, ra57 (2014)