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CELL SIGNALLING

Tyrosine kinases in extracellular space

Despite reports that extracellular proteins are phosphorylated on Tyr residues, there were no known secreted Tyr kinases. Bordoli *et al.* have now identified vertebrate lonesome kinase (VLK; also known as protein kinase domain-containing protein, cytoplasmic) as a secreted Tyr kinase that phosphorylates proteins in the secretory pathway and outside the cell.

The authors searched the literature and existing kinome for a putative secreted Tyr kinase, and VLK emerged as a strong candidate. It has homology to protein kinases while not harbouring the primary sequence that is representative of the established kinase groups. It also has a signal peptide, which is indicative of proteins destined for the secretory pathway. Indeed, assays confirmed that VLK is present in the ER and/or the Golgi in a manner dependent on the putative signal peptide. In addition, full length VLK, but not VLK lacking the signal peptide (VLK^{-SP}), was detected in the conditioned medium of transfected cells. Endogenous VLK is also secreted and this secretion was prevented by the addition of brefeldin A, which inhibits ER–Golgi transport. Thus, VLK localizes to the secretory pathway by its signal peptide and is secreted. Interestingly, VLK kinase activity was necessary for its secretion as VLK proteins with aberrant kinase domains were not found in conditioned media from transfected cells.

To determine whether VLK secretion is physiologically regulated, the authors studied the degranulation (that is, the regulated release of the contents of granules inside cells) of human platelets, in which VLK is highly expressed. Stimulation of platelet degranulation led to the presence of VLK, and proteins that are phosphorylated on Tyr, in the cell supernatant (releasate). Importantly, the addition of ATP to supernatant increased protein Tyr phosphorylation, confirming that extracellular VLK is active. Furthermore, degrading endogenous extracellular ATP that is released from platelet dense granules reduced protein Tyr phosphorylation, confirming that released endogenous ATP can support extracellular kinase activity.

So, what proteins does VLK phosphorylate? Coexpression of VLK with one of five different matrix metalloproteinases (MMPs) resulted in their Tyr phosphorylation on sites previously found to be phosphorylated *in vivo*, and depletion of VLK by shRNA reduced the level of Tyr phosphorylation on MMP13 that was present in conditioned media from transfected cells. VLK, but not VLK^{-SP} (which cannot be secreted), could also phosphorylate several other extracellular substrates. Thus, VLK can phosphorylate extracellular proteins on Tyr. Using mass spectrometry, the authors also identified 140 potential VLK-generated Tyr phosphopeptides, 48 of which belong to proteins with a signal peptide or to the extracellular domain of transmembrane proteins. So, VLK can also phosphorylate endogenous proteins in the secretory pathway.

In sum, this study identifies VLK as the first extracellular Tyr kinase, and finds that it targets extracellular proteins as well as proteins in the secretory pathway. This finding will open new avenues of research, especially in the field of the extracellular matrix.

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> “this study identifies VLK as the first extracellular Tyr kinase”

ORIGINAL RESEARCH PAPER Bordoli, M. R. *et al.* A secreted tyrosine kinase acts in the extracellular environment. *Cell* **158**, 1033–1044 (2014)