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Secreted protein kinases?

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The *Trends in Biochemical Sciences* review 'Secreted protein kinases' portrays FAM20C (family with sequence similarity 20), recently discovered to be Golgi casein kinase (G-CK), as the archetypal member of the four-jointed family of secreted protein kinases that phosphorylate target proteins in the Golgi and in extracellular matrices [1,2]. Although the review is scholarly and interesting, we feel that the existing literature does not support the underlying assumption that these kinases are secreted in physiological quantities, or that any of them serve a necessary function in the extracellular matrix *in vivo*. We believe that the weight of evidence is currently against this being a family of secreted kinases and that additional efforts should be directed at better localization of the kinases before posing and testing hypotheses about their possible roles in extracellular matrices.

FAM20C is one of three members of the FAM20 family, which comprises FAM20A, FAM20B, and FAM20C in mammals. This family was originally discovered in hematopoietic cells and, based upon behavior of the ectopically expressed proteins in cell lines, was described as being a family of secreted proteins [3]. A similar result was obtained for ectopically expressed FAM20C when it was identified as G-CK [1]. However, there are reasons to doubt that the FAM20 kinases are secreted. Immunolocalization of ectopically expressed FAM20A and FAM20B in HEK293T cells localized these proteins to the Golgi, and western blot analyses of the cell extracts and medium detected FAM20A and FAM20B only in the cell extracts [4]. FAM20B is a Golgi kinase that helps regulate glycosaminoglycan synthesis by phosphorylating xylose in the glycosaminoglycan-protein linkage region of proteoglycans [5], which precedes and influences subsequent steps in GAG synthesis (including chain termination) that occur within the Golgi [6]. The known function of FAM20B strongly suggests it could not carry out its function in the extracellular matrix. The evidence supports that the proteins most closely related to G-CK are resident in the Golgi and are not secreted.

The conclusion that FAM20C is secreted rests principally on *in vitro* studies using ectopically expressed protein that may not reflect the situation *in vivo*. FAM20C has no predicted transmembrane domain, so Golgi localization of FAM20C may require interactions with a Golgi transmembrane protein [7]. If G-CK and the interacting proteins that hold it in the Golgi are normally expressed in similar amounts, overexpression of the kinase could exceed the capacity of the cells for retaining the recombinant protein in the Golgi. Cell death in culture releases intracellular proteins into the media and the amount of cells and media compared for relative localization can be arbitrary.

The only *in vivo* evidence cited for possible FAM20C secretion was the detection of G-CK activity in cows' milk [8]. However, in that study it was calculated that the ratio of G-CK in the Golgi relative to milk was 47.7 to 1 and was similar to that of galactosyltransferase; a

Golgi enzyme with no putative extracellular function. They developed a straight-forward two-step fractionation procedure that achieved an 84 000-fold enrichment while retaining 46% of the original G-CK activity, but still did not obtain sufficient protein for identification. Thus, the only *in vivo* FAM20C data are not consistent with a high proportion of FAM20C being secreted *in vivo*, as can occur *in vitro* during ectopic overexpression, and does not support the assumption of the review that FAM20C is secreted and is likely to serve an extracellular function.

Four-jointed (Fj) is the FAM20 homolog in *Drosophila* [9]. Fj is cleaved intracellularly and its C terminus is secreted. It was proposed that its C terminus acts as a signaling molecule. However, elegant studies using transgenic rescues of an Fj mutant phenotype demonstrated that Fj is largely localized to the Golgi *in vivo* and that cleavage and secretion of Fj is not essential for its activity, and may be a mechanism to downregulate Fj activity during normal development [10].

We conclude that the current literature does not support an assumption that the four-jointed family of kinases in mammals are secreted in appreciable quantities *in vivo*, or that they function in extracellular matrices. Testing hypotheses concerning their specific roles in the extracellular matrix is premature and should be preceded by demonstrations that the kinase is actually secreted at levels comparable to that found in the Golgi, and if possible, demonstration that a failure to secrete the kinase results in a loss of function.

References

1. Tagliabracci VS, et al. Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. *Science*. 2012; 336:1150–1153. [PubMed: 22582013]
2. Tagliabracci VS, et al. Secreted protein kinases. *Trends Biochem. Sci.* 2013; 38:121–130. [PubMed: 23276407]
3. Nalbant D, et al. FAM20: an evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. *BMC Genomics*. 2005; 6:11. [PubMed: 15676076]
4. Ishikawa HO, et al. The Raine syndrome protein FAM20C is a Golgi kinase that phosphorylates bio-mineralization proteins. *PLoS ONE*. 2012; 7:e42988. [PubMed: 22900076]
5. Koike T, et al. FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan-protein linkage region. *Biochem. J.* 2009; 421:157–162. [PubMed: 19473117]
6. Nadanaka S, et al. EXTL2, a member of EXT family of tumor suppressors, controls glycosaminoglycan biosynthesis in a xylose kinase-dependent manner. *J. Biol. Chem.* 2013; 10:10.
7. Banfield DK. Mechanisms of protein retention in the Golgi. *Cold Spring Harb. Perspect. Biol.* 2011; 3:a005264. [PubMed: 21525512]
8. Duncan JS, et al. Purification of Golgi casein kinase from bovine milk. *Biochem. J.* 2000; 350(Pt 2): 463–468. [PubMed: 10947960]
9. Ishikawa HO, et al. Four-jointed is a Golgi kinase that phosphorylates a subset of cadherin domains. *Science*. 2008; 321:401–404. [PubMed: 18635802]
10. Strutt H, et al. Cleavage and secretion is not required for Four-jointed function in *Drosophila* patterning. *Development*. 2004; 131:881–890. [PubMed: 14757640]