PACE4 (PCSK6): another proprotein convertase link to iron homeostasis?

We read with great interest the recent article by Pagani *et al.*[1] in which the authors identified a soluble form of transferrin receptor-2 (TfR2) in the media of cultured human erythroid cells and transfected cell lines. Although the authors indicated that the cleavage occurs at the cell surface and is regulated by diferric transferrin,[1] they did not identify the sheddase involved in this process.

We think that the results of study by Pagani *et al.*[1] may help to predict which protease is involved in the TfR2 shedding. Using a general inhibitor of proprotein convertases, decanoyl-Arg-Val-Lys-Arg-chloromethylketone (RVKR-cmk),[2] the authors revealed that a proprotein convertase is implicated in the TfR2 cleavage. Indeed, in HeLa cells transfected with TfR2, this inhibitor treatment resulted in a strong reduction of soluble TfR2 in the media.[1] It is known that the dec-RVKR-cmk inhibitor blocks the activity of the seven basic specific proprotein convertases, i.e. PC1, PC2, furin, PC4, PC5, PACE4 and PC7.[2] But, some proprotein convertases are tissue-specific and can be ruled out, e.g., PC1 and PC2, which are specifically expressed in neural and endocrine cells, and PC4, which is exclusively expressed in germ cells. The remaining members of the family (furin, PC5, PACE4 and PC7) are ubiquitously/widely expressed and share overlapping cellular localizations,[2] but two of them are known to be involved in iron homeostasis. Indeed, furin can cleave hepcidin[3] and hemojuvelin[4] whereas PC7 is the sheddase of transferrin receptor-1.[3] Pagani *et al.*[1] demonstrated that both furin and PC7 are not involved in TfR2 shedding because their overexpression in HeLa cells transfected with TfR2 did not modify the soluble TfR2 release. Consequently, PC5 and PACE4 are the only two proprotein convertases potentially involved in the cleavage of TfR2. However, it is known that HeLa cells express PACE4 but not PC5.[5] Moreover, it was observed that UT7 erythroleukemia cells released soluble TfR2 in the media[1] and that K562 cells, which are also erythroleukemic cells, express PACE4 but not PC5 mRNA.[3] It is also known that TfR2 is mainly expressed in the liver[6] and that PACE4 mRNA levels were approximately 40-fold higher than those of PC5 mRNA in mouse primary hepatocytes.[7] Finally, PACE4 is activated at the cell surface, where it binds heparin sulfate proteoglycans,[8] and hence it cleaves its substrates at the cell surface. Taken together, these data strongly suggest that PACE4 is the proprotein convertase involved in the cleavage of TfR2. Interestingly, analysis of the extracellular domain of the human TfR2 sequence revealed a putative proprotein convertase-cleavage site, **R**QTSL**R**$_{163\downarrow}$ER with an Arg at the P1 (residue 163) and P6 (residue 158) positions.[2] Nevertheless, because PACE4 may activate other proteases, such as the metalloprotease ADAMTS4,[2] we cannot exclude that the TfR2 was cleaved indirectly by activation of a latent protease.

In conclusion, we suggest that PACE4 constitutes the third member of the proprotein convertase family implicated in iron homeostasis probably through TfR2 cleavage but possibly also via the activation of the bone morphogenetic protein-6, a key regulator of iron metabolism,[9] which was reported to be cleaved by PACE4 but not by furin in the mouse embryonic carcinoma-derived cell line ATDC5.[10]

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