Kallikrein Signalling In Cancer Via Proteinase-Activated Receptors

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Proteinase-activated receptors (PARs) are a family of G-protein coupled cell surface receptors that can be activated by proteolytic removal of their extracellular N-terminus to reveal a tethered self-activating ligand sequence (Endocr. Rev. 2005; 26(1):1-43). Three of the four known PARs (PARs 1, 3 and 4) are targeted by thrombin, whereas PAR_2 is activated by trypsin-like proteinases. Human kallikreins (hKs), a family of trypsin-like secreted serine proteinases, have been implicated in many pathological processes related to tumour progression and cell survival (Nat. Rev. Cancer 2004; 4(11):876-90). One member of this family, hK14, has a wide tissue distribution, is differentially expressed in several tumours and has been proposed as a possible biomarker for breast and ovarian cancer. Many in vitro experiments have shown that kallikrein 14 can cleave several substrates associated with tissue remodelling and cancer, such as laminin, alpha-5 and collagen IV. We hypothesized that hK14, as a prototype kallikrein, may modulate cell function by regulating (activating or inactivating/dis-arming) proteinase-activated receptor (PAR) signaling. Therefore, we tested the ability of hK14: (1) to cleave synthetic peptide sequences based on the cleavage/activation motifs of PARs 1, 2 and 4, (2) to activate PAR_1/PAR_2 and PAR_4-mediated calcium signaling in cultured human HEK and rat PAR_2-KNRK cells, (3) to cause PAR-triggered vasorelaxation in vascular tissues from rats and mice and (4) to activate PAR_4 signalling and aggregation in rat platelets. Proteomic analysis of the cleavage products generated by incubating synthetic N-terminal peptides based on PARs 1, 2 and 4 with hK14 identified cleavage sites consistent with tryptic receptor activation, as well as downstream cleavage sites that may cause receptor dis-arming. hK14 activated PAR_2 in cultured cells (calcium signalling) and caused PAR_2-mediated relaxation of rat and murine vascular tissue. In addition, hK14 had a dual action on PAR_1, depending on the enzyme concentration (principally dis-arming, with minimal activation). Importantly, in human platelets, hK14 was able to cause aggregation by activating PAR_4 whilst dis-arming PAR_1. Thus, in the setting of human tumours, known to be platelet-rich, hK14 would trigger platelet aggregation and the preferential release of platelet endostatin rather than VEGF (PNAS 2005; 102(1):216-20). We conclude that in human tumours, kallikrein 14, which signals preferentially via PARs 2 and 4, may play a novel pathophysiological role to regulate tumour growth and metastasis, like matrix metalloproteinase-1, that triggers cell invasion via PAR_1 (Cell. 2005; 11;120(3):303-13). (Supported by the Canadian Institutes of Health Research)