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P2X7-MMP9 pathway in atherosclerosis: set up and characterization of ex-vivo and in vitro human vascular models

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Atherosclerotic plaque rupture with thrombosis is a major cause of cardiovascular events. The natural history of plaque progression is still unknown and the plaque destabilization unpredictable. We have recently suggested that P2X7 purinergic receptor expressed in carotid plaques (CP) might play a role a role in the pathogenesis of atherosclerosis. P2X7 activation by ATP leads to a broad spectrum of effects, including membrane blebbing, tissue factor (TF)-dependent thrombosis in mice, metalloproteases (MMPs) activation and release. MMPs deregulation may alter extracellular matrix network, affect vascular smooth muscle cell (VSMC) migration, contributing to plaque rupture. To study the P2X7 pathway role in MMPs regulation and in CP progression to thrombosis we developed functional models. We setup and characterized an ex-vivo tissue culture model, using human explants from either atherosclerotic (CP) or not (internal mammary artery, IMA) vessels of patients submitted to carotid endoarterectomy or to coronary artery bypass surgery. The preservation of vascular features in ex-vivo culture was verified at different time points (from 24 hours to 15 days) by evaluating the morphology (Hematoxylin/Eosin, Movat's), and the expression of wall cells (aSMA, sm22, Vimentin, CD68, FSP1) and extracellular matrix markers (Collagen type I), of P2X7, MMP9 and TF by confocal microscopy and/or biochemistry. ATP quantification and gel zymography in tissue protein extracts and culture supernatants evaluated the metabolic activity and the MMPs-related gelatinolysis of cultured vessels. A time frame of 4 days was identified as the maximum for structurally and functionally preserved vessel ex-vivo culture; functional loss was seen at day7, morphology alteration at days 10 and 15. P2X7 and MMP9 were previously detected in CP intima-media, thus to perform targeted functional studies we obtained VSMC from ateromasic areas of CP and from IMA. IMA fragments released cells for up to 3 months, CP for up to 2 months. VSMC were characterized similarly to tissue cultures; when freshly-explanted or at passage1, VSMC displayed P2X7, MMP9 molecules expression and gelatinolytic activity. At further passages P2X7 was undetected or unmounted on membrane, MMP9 expression and gelatinolysis absent. Treatment with ATP (50-200mM, 24hours), P2X7 antagonists (KN62, A74003) affected cell metabolism and gelatinolysis but not P2X7 expression. Our data showed that CP and IMA ex-vivo cultures and primary VSMC in vitro cultures represent suitable functional models to investigate the role of P2X7-MMP9 pathway in vascular pathology.