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Dissertations-Kurzfassung

Analysis of the role of stabilin-1 in tumour growth and its functions in tumour-associated macrophages

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Tumour-associated macrophages (TAM) are crucial participants in malignant progression. Acquisition of M2 (alternatively activated) phenotype by TAM during tumour progression makes them unable to exert effective anti-tumour responses, but enhances the immunosuppressive and tumour-supportive properties of TAM including support of tumour invasion, metastasis and angiogenesis. Scavenger/sorting receptor stabilin-1 is a marker of M2 macrophages and was found to be expressed by TAM in several murine tumour models. However, its role in tumour progression was not defined. In order to identify the role of stabilin-1 in tumour progression, the model for mammary adenocarcinoma (TS/A) was established in BALB/c mice with stabilin-1 knockout. Growth of TS/A mammary adenocarcinoma in stabilin-1 knockout (ko) mice was suppressed by 36%. To identify the role of stabilin-1 in TAM biology and to reveal functions of stabilin-1 that are important for tumour progression, isolation of high purity TAM from TS/A murine adenocarcinoma was established and optimized. Flow cytometry quantification revealed that adhesion/internalisation of extracellular SPARC is decreased by approximately 30% in the isolated stabilin-1 ko TAM compared to wt TAM. Immunofluorescent/confocal microscopy analysis showed that transport of SPARC into the endocytic pathway was significantly impaired in the stabilin-1 ko TAM. Using Affimetrix microarrays, Real-Time PCR and Western blotting it was found that TAM from stabilin-1 ko BALB/c mice had impaired expression of PKCbeta on mRNA and protein levels. Real-Time PCR analysis identified that PKCbeta mRNA levels are decreased approximately 6 times in stabilin-1 ko versus wt TAM. Moreover, in HEK293 cells stably transfected with stabilin-1 expression of PKCbeta mRNA was enhanced approximately 10 times compared to the control HEK293-vector cells. The ability of stabilin-1 to mediate activation of PKCbeta promoter has been examined in stabilin-1 stably transfected HEK293 cells transfected with luciferase reporter construct driven by PKCbeta promoter. It was found that presence of stabilin-1 enhances activation of PKCbeta promoter approximately 5 times. In order to identify further functions of stabilin-1 that can contribute to the control of tumour growth, the ability of stabilin-1 to phagocytose apoptotic cells was investigated. It was demonstrated that stabilin-1 expressed on monocyte-derived macrophages participates in the recognition and uptake of apoptotic cells (aged RBCs and apoptotic Jurkat cells). This process was found to be specific, since stabilin-1 was not involved in phagocytosis of fluorescent beads. In conclusion, in the present study it has been demonstrated for the first time that stabilin-1 supports growth of murine mammary adenocarcinoma in vivo. It was established, that impairment of two stabilin-1 mediated functions in stabilin-1 ko TAM correlates with reduced tumour growth: 1) decreased SPARC clearance; and 2) decreased expression of PKCbeta. Moreover, in the present study a new function of stabilin-1 has been identified — clearance of apoptotic cells that can also be related to the stabilin-1 mediated tumour growth support.