Ruprecht-Karls-Universität Heidelberg
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Dissertations-Kurzfassung

Chi3L1 modulates HS-bound cytokines and growth factors by ECM interaction

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Chitinases are highly preserved enzymes ubiquitously present in bacteria, fungi, plants and mammalians. The mammalian chitinase family comprises nine members. In humans, chitinases has been attributed to inflammatory diseases such as arteriosclerosis, or, to tumour progression. The current study investigated the molecular function of the mammalian chitinase-3 like 1 (Chi3L1). Chi3L1 has been shown to induce angiogenesis and cell migration. Although the acting molecular mechanisms are still unclear, the binding to different glycosaminoglycans (GAGs) and type I Collagen might contribute to the biological impact of Chi3L1. The here performed research based on two previously hypothesised molecular pathways. First, it was analysed whether Chi3L1 is translocated from the cytoplasm into the cell nucleus affecting gene transcription. Secondly, it was investigated whether Chi3L1 is able to remodel the extracellular matrix.

Because clinical data suggest a correlation between Chi3L1 plasma levels and the severity of melanoma progression, the impact of recombinant Chi3L1 on the migratory properties of human (BLM) and murine (ret) melanoma cells was tested. Administration of recombinant human Chi3L1 was not able to induce cellular migration. However, the secretome of Chi3L1 overexpressing BLM cells was changed to a pro-migratory and pro-angiogenic profile as indicated by elevated levels of CCL2 and VEGF-A in the supernatant. Conditioned medium of those cells was found to induce the migration of melanoma cells expressing the CCL2 receptor CCR4.

Although overexpression of human Chi3L1 in BLM cells boosted the levels of various pro-migratory and pro-inflammatory cytokines as well as growth factors (e.g. CCL2 and VEGF-A) in the cell supernatant, quantitative real time PCR documented that the transcription of the corresponding genes was not affected. In line with that, immune fluorescence analysis could not confirm the previously postulated nuclear localization of Chi3L1.

Accordingly, the second hypothesis was followed suggesting a potential “soft remodelling” of the extracellular matrix (ECM). It was investigated whether Chi3L1 is able to remodel the extracellular matrix through the release of GAG-stored factors such as VEGF-A or CCL2. To this end, a solid-state ligand binding assay was devised. It was found that physiological concentrations of Chi3L1 of about [100ng/ml] were sufficient to release GAG-bound VEGF-A or CCL2. In comparison to CCL2, the antagonism of Chi3L1 and VEGF-A was significantly more pronounced. Tube formation assays and an ex vivo porcine wound healing model further suggested that Chi3L1 promotes angiogenesis through the release of ECM bound proangiogenic factors.

In conclusion, it was found that Chi3L1 modulates the availability of extracellular matrix retained signalling molecules such as CCL2 or VEGF-A through competitive bind to GAGs. Besides the observed in vitro migration and angiogenic studies, ECM dependent pro angiogenic properties of Chi3L1 were confirmed in ex vivo experiments. Although further functional in vivo experiments are required to consolidate the molecular impact of the here reported findings, it could be postulated that blockage of Chi3L1 may reduce tumour-induced angiogenesis and tumour cell migration.