Conclusions and Perspective

Description

Results gathered in a series of articlesBaranova et al., 2011Baranova et al., 2013Baranova et al., 2014provide novel mechanistic insights into the assembly and remodeling of HA-rich matrices under inflammation and inflammation like conditions.

The quantitative data from the model systems demonstrate that full length TSG-6 forms oligomers upon interaction with HA which act as effective HA cross-linkers. At concentrations that might be physiologically relevant, full length TSG-6 is able to remodel HA films from a highly expanded state into a condensed and rather rigid coat. Such remodeling may occur locally in the endothelial glycocalix and serve as a primary signal for leukocyte attraction. Extracellular matrix remodeling by TSG-6 may also affect mechanical properties of cells and significantly change their phenotype. In arthritis, HA retention by TSG 6 cross-linking in the coat of chondrocytes may contribute to its chondroprotective function.

I?I dictates TSG-6 activity and remodels HA matrix properties. TSG-6 induced cross-linking and compaction of HA films is impaired in the presence of I?I. This leads to the inhibition of the TSG-6-mediated HA binding to CD44 positive cells. Prolonged incubation with TSG-6 and I?I results in HA films that contain, in addition to covalently HA-bound HCs, several tightly but non-covalently bound molecular species. The non-covalently bound material, which included TSG-6, has the ability to transfer HCs onto HA.

These results also demonstrate that the encounter between the proteins TSG 6, I?I and PTX3 determines the structural and morphological properties of the HA matrix. PTX3 is inert to binding to an HA matrix that results from the ternary interaction of I?I, TSG-6 and HA, even though such matrices contain HA•HC complexes which have previously suggested as a potential PTX3 ligand. Moreover, PTX3 cannot be incorporated into an HA matrix via TSG-6 alone. Instead, we show that the interaction of PTX3 with I?I and TSG-6 prior to encounter with HA is required for efficient incorporation into the matrix. PTX3 seems to be involved in the formation of cross-links. Even though we succeeded to incorporate all proteins that have been found to be crucial for COC matrix stability into HA films, the exact structure of the cross-linking nodes in the HA matrix remains unknown. Further studies with selected protein domains (in particular recombinant HCs) or mutant forms (for instance PTX3 that forms dimers or tetramers) using the assays developed in this thesis should allow to shed further light onto this question.

Based on the above-listed findings, it can be hypothesized that there is a functional interplay of different cross-linking mechanisms in the assembly of the COC matrix. In particular, TSG-6 induced cross-linking might play a role at the early stages of COC matrix assembly, when the synthesis of HA and TSG-6 is elevated. TSG 6 could help to retain overproduced HA in the matrix of cumulus cells before the follicle wall becomes permeable for another TSG-6 ligand – I?I. When I?I enters the follicle, TSG-6 cross-linking would be impaired. To stabilize the entire structure, PTX3 incorporates into the HA matrix at regions where all proteins (or their subunits) encounter each other. This would lead to the formation of cross-links which are transient and thus allow incorporation of new HA chains and

ultimately COC matrix expansion.
In the future, this hypothesis can be verified in three-dimensional (3D) model systems. To this end, HA-coated microbeads could be embedded into an HA matrix by external addition of proteins and HA. The mechanical properties of the artificial matrices at different compositions can then be probed and compared to a real COC matrix. The reconstitution of such an extended 3D material should provide a valuable test if HA, TSG-6, I?I and PTX3 together can indeed compose a minimal system required for the assembly, expansion and stabilization of COC matrix.

Category

1. News