Poster Number

49 **Phylogenetic Analysis of Planarian Collagens and their Roles in Regeneration**

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Stem cells are regulated by the microenvironment or niche they reside in, which consists of growth factors, niche cells and the extracellular matrix. The ECM acts as both a structural component and as a reservoir for growth factors that are released upon degradation. During regeneration, stem cells in the planarian are activated to migrate and proliferate; however, the role of the ECM in stem cell regulation is still unclear. Analysis of an EST library of planarian transcripts revealed nine fibrillar-related collagen chains (DjCol1-9). Sequence and structural analysis reveal interuptions in the triple helical domain, the functions of which are unknown. Bioinformatic and phylogenetic analysis reveal that the fibrillar A and B clades are present in the planarian. Proteomics analysis of the blastema of the planarian Dugesia japonica showed that DjCol1, DjCol2, DjCol4 and DjCol6 are dynamically upregulated. Djcol1 and -2 are co-expressed with piwi expressing stem cells in the intact worm but are not co-expressed in stem cells of the regenerating blastema. This is an interesting collagen expression pattern in stem cells, suggesting a potential functional relationship in cell maintenance and regeneration. Indeed, knockdown of Djcol1 and -2 by RNAi showed increased number of piwi expressing cells in the regenerating blastema and evidence of enhanced regeneration capacity. On the other hand, Djcol3 and -4 are expressed in supepidermal differentiated cells, and are expressed slightly later during regeneration, suggesting a differing role in the regeneration process. Djcol5 also shows similar expression pattern to the other four collagens. This study provides the first insight into the role of ECM in the regulation of stem cell property/function, with collagens as a negative regulator.

50 **The role of extracellular matrix in planarian regeneration**

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As an important niche component, the role of extracellular matrix (ECM) in stem cell biology is well recognized, however, its role in tissue regeneration is not well understood. Planarians are able to regenerate any missing parts of the organism and this feat is thought to be contributing by its large population of stem cells, which are distributed throughout the inner mesenchymal region. Here we use planarian as the model system to study the dynamic protein expression changes during tissue regeneration in order to gain insights into the role of ECM in regeneration.

Using a novel time-lapse microscopy, I demonstrated that the process of planarian blastema development is coordinated with the proliferation and differentiation of stem cells and also the reorganization of tissue components. Proteome expression profiling of the blastema during the first 4 days of regeneration revealed that many ECMs were dynamically expressed during this process suggesting the regulation of matrix assembly and remodeling is important.

Expression analysis of two collagen chains, renamed Dj-col1 and Dj-col2 showed that they are co-expressed with piwi expressing stem cells in intact planarians; however, in blastema, their co-expression with piwi positive cells become delinked. Knock-down them by RNAi amputated planarians showed “faster regeneration”. Quantitative analysis indicated there was a general increase in the number of piwi expressing stem cells following RNAi treatment. Together, these findings demonstrated these collagen chains acts as negative regulators of stem cells and regeneration and provide the first insight into the role of ECM in the regenerative processes of planarians.