Extracellular Matrix Perturbation in Down Syndrome Pathology

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The mechanisms by which the excess genetic material of chromosome 21 results in the dysmorphologic features of Down syndrome (DS) are largely unknown. A reason of the specific phenotypes of the individuals could originate from a perturbation of extracellular matrix (ECM). Collagen type VI (COLVI) is an ECM component that has three chains, *α1* and *α2*. Each chain contains a short triple-helical region and globular extensions at the N and C terminus. COLVI molecules associate laterally in an antiparallel fashion into dimers that are stabilized by disulfide bridges. The dimers aggregate further into tetramers that are secreted into the extracellular matrix, where they join end to end into microfibrils. COLVI is thought to participate in cell adhesion and migration through interaction with member of the integrin receptor family or the NG2 proteoglycans. The genes that encode the *α1* and *α2*chains of COLVI, are located in chromosome 21, though for the chain *α3,* the gene is located in chromosome 2. An extra chromosome in DS could result to differences in the quantity of collagen VI, altering also its physiologic properties.

Hyaluronan (HA) is a component of the connective tissue and is present in the ECM of most vertebrate tissues. It plays an important role in several cellular events, such as proliferation, migration and adhesion, whereas it is implicated in many morphogenetic processes during vertebrate embryogenesis [1]. Perturbations in hyaluronan levels can lead to dramatic changes in tissue homeostasis. An alteration in its metabolism could also be involved in the pathogenesis of several structural defects of DS.

Wharton's jelly (WJ), the ECM of umbilical cord (UC), is composed of an insoluble fibrillar network of different collagen types, within which soluble open coil polysaccharides are held. Of these, the predominant one is HA and a smaller part (30%) is formed by protein linked sulphated glycosaminoglycans (GAG). Although HA has been shown to play a crucial role in embryogenesis and tissue remodelling, limited information is available on the hyaluronan UC-ECM regulation during pregnancy. Moreover, several studies have demonstrated that the composition of the UC-ECM is influenced by a number of conditions occurring during pregnancy, such as preeclampsia, diabetes, intrauterine growth restriction and aneuploidy. In particular, nuchal skin biopsies of trisomy 21 fetuses have shown that the dermis and subcutis is richer in HA compared to that of healthy fetuses. Interestingly, it has been shown that HA interacts with COLVI [2]. Therefore, we have decided to investigate the HA synthesis in normal and Down syndrome human UCs during pregnancy. In order to verify this phenomenon and to study the functional role of both COLVI and HA in the ECM, we decided to use human skin fibroblasts of healthy and DS individuals.

Tissue distribution of HA and collagen type VI was assessed by immunohistochemistry using biotinilated-HA-binding protein (bHABP) and monoclonal antibody against collagen type VI (COL6A1/A2). The gene expression of COL6A2, COL6A3, the three hyaluronan synthases and the hyaluronidases (HYAL-1 and HYAL-2) was studied using the real time PCR. HA content was measured by fluorophore assisted carbohydrate electrophoresis (FACE) after specific enzymatic digestions and derivatization, and the molecular size was measured by gel filtration chromatography (FPLC). Human skin fibroblasts obtained from healthy and with DS individuals were cultured in DMEM medium enriched with high concentration glucose, 1% glutamine, 1% antibiotics and 10% fetal bovine serum, in a 95% humidified and 5% CO_2 environment. Experiments were performed when cells reached the confluence. Cells were lysed in urea buffer and protein extracts were analyzed using the SDS-PAGE and the western blot techniques. For the identification of collagen type VI, antibodies against the *α1* and *α2* chains were used. The HA of the conditioned media was analyzed as described in the tissue extracts.

To allow comparison, normal and DS UCs were matched for gestational age. Immunohistochemistry revealed that DS UC was richer in collagen type VI and that in normal UC HA was present mostly in the subamniotic zone, while in DS UC HA was more homogenously distributed from the subamniotic zone to the UC vessels. Real time PCR results suggest that COL6A2 is more expressed in DS UC than in normal. Additionally, a higher expression of HAS 2 and a lower expression of HYAL 2 were found in the Wharton's jelly of DS fetuses in comparison to that of euploid fetuses. Finally, the HA content of DS UC was significantly increased compared to normal UC, as expected from immunohistochemistry and real time PCR experiments. FPLC results showed, additionally, a significant portion of HA molecules with reduced molecular size.

Interestingly, the above results were in accordance with the results of cell culture analysis. More specifically, western blot analysis of the *α1* and *α2* chains of COLVI obtained from the total cell lysate showed a higher amount in the trisomy 21 cell lines, confirming the data obtained from human UC analysis. Additionally, Real-Time PCR showed a higher gene expression of COL6A2 and consequently a higher ratio of COL6A2/COL6A3. Using the FACE analysis, we demonstrated the elevated level of HA production in the trisomic cells.

To sum up, our results showed an elevated amount of HA and COLVI in human UC and skin fibroblasts of foetuses and individuals, respectively, with DS. We observed additionally a higher gene expression of COL6A2, suggesting the gene dosage effect obtained from the extra copy of the chromosome 21. The interaction of HA with COLVI and the perturbation of COLVI in the ECM could explain the increase production of HA.

REFERENCES

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