PROCEEDINGS OF THE XXXIII NATIONAL MEETING OF THE ITALIAN SOCIETY FOR THE STUDY OF CONNECTIVE TISSUES (SISC)

Pavia, October 3-4, 2013
Aula Magna Collegio Cairoli
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European Journal of Histochemistry

a journal of functional cytology

The European Journal of Histochemistry was founded in 1954 by Maffo Vialli and published until 1979 under the title of Rivista di Istochimica Normale e Patologica, from 1980 to 1990 as Basic and Applied Histochemistry and in 1991 as European Journal of Basic and Applied Histochemistry. It is published under the auspices of the University of Pavia and of the Ferrata Storti Foundation, Pavia, Italy.

The European Journal of Histochemistry is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC).

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invited lectures

EPITHELIUM/STROMA CROSS-TALK IN BREAST CANCER

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During tumorigenesis stroma initially plays a suppressing role, but overtime evolves to promote tumor growth, as demonstrated in many experimental studies. Normal tissue architecture becomes disordered; the extracellular matrix (ECM) is remodeled by mesenchymal cells and the relationships between epithelium and basement membrane are disrupted.

Tumor stroma is the microenvironment which surrounds the malignant cells and makes up about a half of malignant tumors. The cells found in the stroma include blood and lymph endothelial cells, pericytes, leukocytes, macrophages and fibroblasts. In particular, fibroblasts termed “cancer associated fibroblasts” (CAFs) have received increased attention because of their involvement in tumor development, including invasion and metastasis. In the tumor stroma, a great number of CAFs and myofibroblasts, high capillary density, deposition of mature type I collagen and other ECM components are observed.

The growing role of the reciprocal interaction between epithelial and stromal cells in the development and progression of breast cancer has been recognized. To verify if this cross-talk may affect some aspects of cell biology (i.e. proliferation, adhesion molecule expression, membrane fluidity, migration), we co-cultured estrogen cancer cells with fibroblasts isolated from breast healthy (MCF-7) or ER-negative, highly invasive and metastatic (MDA-MB-231) breast cancer cells with fibroblasts isolated from breast healthy or from breast tumor stroma (CAFs) in monolayer or in a three-dimensional system (nodules). In terms of proliferation, we demonstrated that CAFs exert different effects on both ER+ and ER- breast cancer cells, while mammary cancer cells had no influence on the growth of both kinds of fibroblasts. NFs and CAFs respectively induced or inhibited E-cadherin expression in MCF-7 cells; CAFs promoted N-cadherin up-regulation in MDA-MB-231 cells and its de novo expression in MCF-7 cells. An increase in N-cadherin was observed in CAFs but not in NFs, as a result of the interaction with both kinds of cancer cells. Plasma membrane labeling of monolayer cultures with the fluorescent probe Laurdan showed an enhancement of the membrane fluidity in cancer cells co-cultured with NFs or CAFs. An increase in lipid packing density of fibroblast membranes was promoted by MCF-7 cells. Time-lapsed cell tracking analysis of mammary cancer cells co-cultured with NFs or CAFs revealed an enhancement of tumor cell migration velocity and a marked increase in the directness induced by CAFs.

A dramatic increase in Stearoyl-CoA desaturase 1 (SCD1), the main enzyme regulating the fatty acid membrane composition, has been observed in both MCF-7 and MDA-MB-231 cells as the result of the interaction with CAFs. In addition, the presence of fibroblasts, in particular CAFs, stimulated binding of SREBP1, a transcription factor which specifically binds to the SREBP response element on the SCD1 promoter, in tumor cells.

Finally, performing co-culture assays in the presence neutralizing antibodies against hepatocyte growth factor, transforming growth factor-beta and fibroblast growth factor-2, which are released by CAFs, we demonstrated that both the speed and the directness increases induced by CAFs in cancer cells migration were strongly reduced or completely abolished.

All these results provide new insights in the understanding the role of CAFs in promoting the tumor cell invasiveness and may help in defining new therapeutic targets in breast cancer.

Osteopetrosis (OP) is a genetic inherited disorder caused by defects in osteoclast formation and function. On the basis of mode of inheritance, we distinguish the Autosomal Dominant Osteopetrosis (ADO, also known as Albers-Schonberg disease) usually considered an adult-onset and benign form characterized by multiple spontaneous fractures and frequent osteomyelitis, and the Autosomal Recessive Osteopetrosis (ARO), also named malignant infantile osteopetrosis because of the severity of the disease leading to death early in life. The first form was identified in 1983, when Carbonic Anhydrase 2 (CA2) gene was found to be mutated in a peculiar form of OP characterized by renal tubular acidosis and cerebral calcification. More recently, the molecular analysis has revealed the genetic heterogeneity of the disease. To date, seven genes have been identified in human ARO forms. The majority of them cause defects in osteoclast function and can be cured by hematopoietic stem cell transplantation (HSCT) since osteoclast originate from myeloid progenitors. However, we have recently identified an OP form caused by defects in TNFSF11 (RANKL), a gene expressed by mesenchymal cells (including osteoblasts and stromal cells). The product of the TNFSF11 is a cytokine RANKL, which acts as a membrane bound factor and soluble molecule and is responsible for fusion of osteoclast progenitors. Our group has demonstrated that RANKL replacement in the RANK1 knockout mouse, might represent a valid treatment for patients carrying defects in the RANKL gene. Indeed, the administration of appropriate doses of RANKL (1 mg/kg every 48 h for 1 month) for a finite period results in dramatic improvement of the trabecular space within bone and in parallel ameliorates the morphology of hematopoietic organs (and unpublished observation). Thus these preclinical data indicate the need of a pilot clinical trial to confirm the efficacy of this treatment in the human disease. Conversely, for patients with ARO forms, the search for a bone marrow donor should be started early in life since HSCT should be performed before the development of irreversible secondary complications, such as visual impairment. To this end, prenatal diagnosis and/or collaboration among multiple clinical disciplines is crucial to define the molecular diagnosis and establish the appropriate therapy. Finally, recent data in literature indicate the feasibility and partial efficacy of gene therapy approach using gammaretroviral vectors in the Tcirg1(oc/oc) mouse model and CD34+ cells obtained from osteopetrotic patients. 1-3.

Bone metastasis is a frequent complication of advanced breast cancer. Fortunately, not all the breast cancers, even with the same diagnostic profiles, will metastasize to bone. This is an indirect evidence of the heterogeneous profile of breast cancer cells of the same origin, among and within the primary tumors. Indeed some cancer cell subpopulations should preferentially express adequate properties that enable them to migrate and grow into the bone matrix, which reciprocally provides a suitable microenvironment for the survival and growth of these cells. The mechanisms underlying the osteotropism of certain cancer cells are complex and involve a number of signals and factors, still not completely clarified. With this aim, we have undertaken the implementation of an in vitro system for the study of the differential proteomic profiling of the bone seeding breast cancer cells.

Viable bone fragments, deriving from surgery on traumatic lesions of young subjects were provided by the orthopaedic team at the DICHIRONS Department, after informed consent of the donor.

Bone fragments, not larger than 0.5 cm, were washed from bio-contaminants under sterile conditions and placed into cell culture capsules with DMEM medium supplemented with foetal bovine serum (10%), L-glutamine (1%), L-ascorbic acid (50 µg/mL) and penicillin/streptomycin (1%). The explants were demonstrated that suppression of HAS2 and HYAL2 was further increased by both stromal and tumour cell. Fibroblasts co-cultured with each tumour cell line using the Transwell system showed that MCF-7 cells induced the further increase HA synthesis by both stromal and tumour cell. Therefore, the aim of the present project is to explore the functional properties of the Q7z3e2 protein and the role it may have in the control of HA synthesis and in cross-talk between the two breast cancer cell lines: MCF-7 (ER positive, low metastatic) and MDA-MB-231 (ER negative, high metastatic) and stromal cells. The Q7z3e2 protein and gene expressions were higher in MCF-7 than MDA-MB-231 cells and mainly localized around or within the nucleus, even though the protein was identified also Q7z3e2 in breast cancer cells and co-culture with fibroblasts demonstrating a cross-talk between these cells. However, silencing of Q7z3e2 in breast cancer cells and co-culture with fibroblasts demonstrated that suppression of HAS2 and HYAL2 was observed only with MDA-MB-231. These data suggest that Q7z3e2 is possibly able to trigger some particular signal pathways inside the cells to regulate the expression of different genes involved in the metabolism of hyaluronan.


PROTEINS INVOLVED IN THE INTERACTION OF BREAST CANCER CELLS WITH TYPE V COLLAGEN

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In the complex scenario of the ECM remodeling at the tumour-stroma interface, the collagens play a crucial role, acting not only as a supporting scaffold for various cell types, but also interacting with the cancer cell surface which may participate to different signaling pathways (cell motility, cytoskeletal remodeling, cell cycle progression, metabolism, etc). Previous researches in our group have focused on the study of interac-
PROTEOMIC EFFECTS INDUCED BY MICROENVIRONMENT COMPONENTS ON 8701-BC BREAST CANCER CELLS: AN UPDATED REPORT

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Many solid tumors, including breast cancer, are composed of heterogeneous cell populations, namely neoplastic cell subpopulations and host cells of various origin, which may interact between each others in complex networks. Tumor cells can modify the epigenetic program of stromal cells that in turn may produce epigenetic changes in tumor cells. The activated stroma, mainly composed of cancer-associated fibroblasts (CAFs), macrophages (TAMs), myofibroblasts and endothelial cells support the aberrant epithelial-mesenchymal cross-talk. At the same time, during tumor progression, the extracellular matrix (ECM) around and within the primary tumor undergoes a remodeling process where several components of the matrix are deposited, degraded or otherwise modified. In addition, intratumoral hypoxia, deriving from high cell density, induce cellular adaptation to low oxygen tension and nutrient deprivation. The new and dynamic microenvironment generates signals that may influence the invasive behaviour of tumor cells through the expression of undefined set of genes, difficult to predict a priori and to study individually. Here we report an updating of our researches on the possible epigenetic influences exerted by selected microenvironment components and hypoxia on tumor cell growth and spreading, evaluated by the proteomics approach. We have used in an vitro model of human breast cancer cells (8701-BC) exposed to different collagen substrates1, decorin2, fibroblasts3, endothelial cells and to a low O2 tension4. Interestingly, while the hypoxia per se did not alter significantly the proteomic expression of 8701-BC cells, the other microenvironment components induced detectable changes in cell morphology and in cell proliferation rate, as well as in some protein clusters (cytoskeleton, metabolic enzymes, apoptosis), suggesting that the host environment influence positively and/or negatively the processes of viability/proliferation and motility, which may contribute in directing the neoplastic cells towards a more or less aggressive phenotype.


DIFFERENTIALLY EXPRESSED PROTEINS IN FIBROBLASTS FROM Abcc6-/- AND Abcc6+/- MICE HIGHLIGHT THE ROLE OF A LOCALLY ALTERED PHOSPHATE METABOLISM IN THE OCCURRENCE OF ECTOPIC CALCIFICATION

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Progressive calcification of elastic fibers is typical of Pseudoxanthoma elasticum (PXE), a rare genetic disease due to mutations in the ABCC6 gene. The pathogenesis of mineralization is only partially known.1 Previous studies on dermal fibroblasts from PXE patients demonstrated that the calcification process is associated to impaired carboxylation of matrix-gla-protein that, in its active form, binds to calcium, therefore inhibiting mineralization.2 However, the recent observation that PXE fibroblasts exhibit also a significant upregulation of alkaline phosphatase (TNAP) activity suggested that an abnormal phosphate metabolism may take place within soft connective tissues, thus contributing to ectopic calcification.3 To improve the understanding of PXE it was developed a transgenic mouse model by specifically inactivating the Abcc6 gene.4 Consistently, Abcc6-/- mice recapitulate several histopathological findings typical of PXE, including the slow progression of the disease.5 Aim of the present study was to isolate fibroblasts from Abcc6-/- and Abcc6+/- mice of different ages (i.e. before and after the development of ectopic calcification) and to investigate proteins controlling phosphate levels in the extracellular matrix. Results demonstrate a down-regulation of pyrophosphatase/phosphodiesterase 1, of progressive ankylosis protein and of osteopontin, whereas bone morphogenetic protein 2 and TNAP activity were up-regulated in fibroblasts from Abcc6-/- animals. These data support the hypothesis that in PXE the unbalanced ratio between factors locally controlling both calcium and phosphate homeostasis are crucial in triggering tissue calcification.

Work supported by grant from FCRM-EctoCal.

are recognized as differentiation markers.

In fact, it is well known that carbohydrates are directly implicated in recognition processes, including adhesion between cells, adhesion of cells to the extracellular matrix, and in specific recognition of cells by one another. In addition, carbohydrates have a significant impact in the tissue repair.2

Methods for chemoselective collagen matrix modifications

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Collagen is widely used as a biomaterial for several tissue engineering applications.3 Carbohydrates are hydrophilic molecules for material surface functionalisation, since they can imbriate the surface with double achievements at once. The first one is given by their polyhydroxylated nature, that confers hydrophobic features to the surface they are linked to. The second one, is due to their ability to convey biological information.2

With respect to the limited lifespan of glutaraldehyde(GA)-treated bioprostheses to date there is almost no alternative when heart valve replacement surgery is required and most advanced current research attempts to develop tissue engineered valve scaffolds to be implanted in vivo or after in vitro preconditioning and dynamic seeding with host cells. However the clinical outcomes of detergent-based cell-depleted tissue engineered xenogeneic constructs are still controversial. Therefore, we investigated whether the clinical drawbacks of GA-treated and decellularized bioprosthetic devices might be related to residual xenogenic epitope and/or to biocompatibility problems associated with by-products of the detergent-based decellularization process. Accordingly we determined the residual content of detergents and that of residual xenogenic antigens in both GA-treated and detergent-based preparations (five different procedures).

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Collagen is the most abundant protein in the body and a major component of the extracellular matrix (ECM); it has been widely used both in vitro and in vivo and has been shown to have suitable properties for tissue engineering applications. One emerging feature is collagen glycosylation, reported as relevant in determining physiological and pathological states. In fact, glycosylated collagen is deeply involved in remodelling and breakdown of ECM; furthermore O-glycosylation events provide a novel regulatory level in the dynamic balance between collagen deposition and turnover with several implications in healthy and pathological processes.1 In addition, it has recently been shown that lectin domains interacts directly with glycosylated collagens,2 modulating the endocyctic efficiency of the receptor toward highly glycosylated collagens such as basement membrane collagen IV. Despite the relevance of glycosylation in eliciting specific biological responses, the chemoselective surface modification of collagen-based scaffolds by carbohydrate epitopes has never been proposed to date.

We have approached the immobilization of simple carbohydrate structures to collagen through the thiol–ene click chemistry, planning the reaction between alkene-derived monosaccharides and thiol-terminated collagen patches, generated via functionalisation of the lysine residues. The presence of monosaccharides introduced by thiol-ene reaction on the patch surface was confirmed by synchrotron analysis (SR-XPS). In order to assess if the exposed monosaccharide could also exploit its biological signalling functions upon recognition of its complementary receptor, preliminary Enzyme Linked Lectin Assays (ELLAs) were performed on the glycosylated collagen samples. Preliminary in vivo biological assays in osteo-arthritis mice clearly indicate that surface modification of collagen patches, even with small biomolecules such as biologically relevant monosaccharide (i.e. glucose and galactose), has a significant impact in the tissue repair.2

Acknowledgment. We gratefully acknowledge Fondazione Cariplo, grant no 2010-0378, and MIUR, under project PRIN 2010/L9SH3K.
**AGE- AND DIABETES-RELATED NONENZYMATIC CROSSLINKS IN COLLAGEN FIBRILS: CANDIDATE AMINO ACIDS INVOLVED IN ADVANCED GLYCATION END-PRODUCTS**

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**Ageing and diabetes share a common deleterious phenomenon, the formation of Advanced Glycation Endproducts (AGEs), which accumulate predominantly in collagen due to its low turnover. Though the general picture of glycation has been identified, the detailed knowledge of which collagen amino acids are involved in AGEs is still missing. In this work we use an atomistic model of a collagen fibril to pinpoint, for the first time, the precise location of amino acids involved in the most relevant AGE, glucosepane. The results show that there are 34 specific lysine-arginine pairs that, due to their relative position and configuration, are likely to form glucosepane.**

**Acknowledgment.** This work has been supported by Fondazione Cariplo, grant n° 2011-0270. High-performance computing resources have been provided by CINECA Consortium through the ISCRA initiative and by DEISA Consortium through the PRACE initiative.


**TENDON SHEATHS AND WATER IN TENDON BIO-MECHANIC**

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In tendon the major tissue component is water representing the 55-70% of the total wet weight. Four groups of tissue water have been described: a) the structural water, b) the bound water, c) the free water and d) the water in transition. In the extracellular matrix of tendon insoluble fibrillar collagen mainly consists of type I collagen which represents the 80-90% of tissue dry weight. Small proteoglycans like decorin and a small amount of biglycan fill inter fibrillar spaces, whereas large proteoglycans like aggrecan and versican seem to be located in inter fibre spaces. Collagen fibers seem to retain the main part of interstitial bound water whereas proteoglycan aggregates are supposed to retain large amount of free water. Fluids move with...

**INHIBITION OF NF-kB ACTIVATION BY β-ARRESTIN 2 IN IL-1 β-INDUCED INFLAMMATION IN MOUSE CHONDROCYTES**

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Hyaluronan (HA) degradation occurring during pathological state produced small HA oligosaccharides that may induce an inflammatory response by interacting with the toll-like receptor 4 (TLR-4) and CD44. TLR-4 stimulation activates different pathways that culminate with the activation of the transcriptional nuclear factor kappaB (NF-kB). The translocation of NF-kB into the nucleus may induce a pathway that involve the transcrip-tional nuclear factor kappaB (NF-kB). The translocation of NF-kB into the nucleus may induce a pathway that involves the transforming growth factor activated kinase-1, (TAK-1), that in turn primes the expression of different inflammatory mediators. β-arrestin 2 is an adaptor protein that alter signalling of G protein and seems to be involved in the down-regulation of the inflammation response.

In this study we investigated the role of β-arrestin 2 in mouse chondrocytes exposed to interleukin-1β. Chondrocyte stimulation with IL-1β enhanced HA levels and its degradation, up-regulated cyclic adenosine monophosphate (cAMP), β-arrestin 2, TAK-1, protein 38 mitogen-activated protein kinase (p38MAPK), and protein kinase A (PKA) mRNA expression and of the related protein levels.

IL-1β treatment also induced NF-kB activation, with a consequent transcription of pro-inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS). The treatment of IL-1β-stimulated chondrocytes with β-arrestin 2 inhibitor, significantly increased the inflammatory response, while the treatment with a specific TAK...
COLLAGEN MATRICES NEOGLYCOSYLATION VIA LYSINE SIDE-CHAINS AND ITS BIOLOGICAL EVALUATION

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Site-selective chemical modification of collagen matrices requires an efficient and chemoselective reaction. Different amino acid side-chains can be exploited toward this aim (cysteine, glutamine, serine, threonine, proline, hydroxyproline, lysine, and arginine). Performing residue-specific chemical reactions on collagen and maintaining its integrity is not an easy task. In the present work lysine amino groups have been functionalised via reductive amination with different glycan structures, obtaining a “neo-glycosylated collagen”. In order to assess the effect of the “neo-glycosylation” reaction on collagen structure, molecular dynamics simulations, AFM, water contact angle and FTIR have been performed. In order to assess if the exposed saccharide could also exploit its biological signaling functions upon recognition by its complementary receptor, preliminary Enzyme Linked Lectin Assays (ELLAs) was performed on the glycosylated collagen samples. Finally, preliminary in vitro biological assays on MG-63 cell lines showed that proliferation of MG63 cell lines on neo-glycosylated collagen is favored if compared to pristine collagen.

Acknowledgment. We gratefully acknowledge Fondazione Cariplo, grant n° 2011-0270, and MIUR, under project PRIN 2010/LY9H3K.


EFFECTS OF NEOGLYCOSYLATED COLLAGEN ON NEURONAL DIFFERENTIATION

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Glycans participate in neural cell migration, neurite outgrowth and synapse formation in both the developing and adult nervous system. Understanding their functions would improve the knowledge in the mechanisms that determine nervous system differentiation and regeneration and could lead to the use of glycans as therapeutic strategies for neurological disorders.

For this reason we studied the effects of native and neoglycosylated collagen (gic-collagen) on neuronal differentiation by using a neuroblastoma, dorsal root ganglioneuroma-derived cell line (F11). The choice of cells was motivated by their capability to differentiate into neurons when exposed to differentiating agents. We compared the behaviour of cells plated on native and gic-collagen with cells seeded on petri dishes. After 7 days, the electrophysiological properties of cells were analysed by the patch-clamp technique in the whole-cell configuration. Sodium current densities showed the tendency to increase from petri dishes to collagen and to gic-collagen and significantly higher.
through the internal compartment to the plasma membrane, even can indicate a regulatory point during the enzyme transport not sufficient condition for the production of HA. The ER stress overexpression of the synthetic enzymes is a necessary but the use of the only free cholesterol or oxysterols, indicating that stress markers (CHOP and GRP78), condition present also with associated with higher expression if endoplasmic reticulum delivered to AoSMC did induce increased HAS expression but Nevertheless, free cholesterol (20 µg/mL) alone or oxysterols the role of sterols in the pro-atherosclerotic transformation. abolishing the HA synthesis and HASs overexpression, highlight- transcription and activation, we depleted oxLDL of cholesterol, that glc-collagen is an efficient material to be employed for neuronal differentiation. Acknowledgment. We gratefully acknowledge Fondazione Cariplo, grant n° 2010-0378, and MIUR, under project PRIN 2010/L9SH3K.


OXIDIZED LDL REGULATE HYALURONAN SYNTHASES ACTIVITY IN HUMAN AORTIC SMOOTH MUSCLE CELLS

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Atherosclerosis, usually classified as a chronic inflammatory disease of the blood vessel wall, is the most common cause of cardiovascular diseases. The localized inflammation causes the thickening of the arterial wall due to the increased deposition of extracellular matrix (ECM) and native LDL trapped within the arterial wall undergo modifications like oxidation (oxLDL). The thickening of the vessel in response to high LDL levels is a hallmark of atherosclerosis, characterized by increased hyaluronan (HA) deposition in the neointima. We investigated the link between internalization of oxLDL and HA production in vitro, using human Aortic Smooth Muscle Cells (AoSMC), showing the effects on scavenger receptor LOX-1 up-regulation and Hyaluronan synthases (HASs) expression. OxLDL treatments enhanced transcripts of HA synthase HAS2 and HAS3 1.9 and 3.8 folds. Accumulated HA stimulated AoSMC migration and monocyte adhesion/ECM. The effects induced by oxLDL were inhibited by blocking LOX-1 scavenger receptor with a specific antibody (10 µg/mL). To unravel the mechanism of HASs transcription and activation, we depleted oxLDL of cholesterol, abolishing the HA synthesis and HASs overexpression, highlighting the role of sterols in the pro-atherosclerotic transformation. Nevertheless, free cholesterol (20 µg/mL) alone or oxysterols delivered to AoSMC did induce increased HAS expression but not HA synthesis. In oxLDL treatments, HA deposition was associated with higher expression if endoplasmic reticulum stress markers (CHOP and GRP78), condition present also with the use of the only free cholesterol or oxysterols, indicating that the overexpression of the synthetic enzymes is a necessary but not sufficient condition for the production of HA. The ER stress can indicate a regulatory point during the enzyme transport through the internal compartment to the plasma membrane, even though is possible a finest regulation at transcriptional level involving microRNA or antisense RNA.


SEA URCHINS AND MECHANICALLY ADAPTABLE CONNECTIVE TISSUES: ALTERNATIVE SOURCES FOR BIOMATERIAL DESIGN

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Sea urchins, like all echinoderms (starfish, sea cucumber, etc.), possess connective tissues that undergo drastic changes in their mechanical properties (Muttable Collagenous Tissues: MCTs). Mammalian connective tissues rarely undergo significant changes within a physiological timescale, the only major exception being the destabilizing and restiffening of the mammalian uterine cervix at the end of pregnancy. In contrast, MCT can switch reversibly between stiff and compliant conditions in timescales of less than a second to minutes following nervous stimulation. In view of this behaviour, MCT could be an inspiration for new matrices capable of changing their molecular and structural conformation in response to external stimuli. Furthermore, elucidating the molecular mechanism underlying MCT mutability could have implications for veterinary and biomedical science, particularly regarding the pathological plasticization or stiffening of connective tissue structures. The MIMESIS (Marine Invertebrate Models & Engineered Substrates for Innovative Bio-Scaffolds) project has been developed within this scientific context. This contribution presents a review of the distinctive features of MCT together with the first results regarding the production of MCT-derived matrices as cell culture /tissue regeneration substrates.


CHARACTERIZATION OF ZEBRAFISH COLLAGEN TYPE I

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Procollagen type I is the major constituent of connective tissues. In higher vertebrates it is a heterotrimer formed by two proα1 and one proα2 chains folded in a triple helical superstructure characterized by the presence of N- and C-terminal random coiled propeptides. In the last years the bony fish D.
form in human megakaryocytes. However, little information on the expression of the Extra Domain A (EDA) fibronectin isoform in activated fibroblasts that contains variable proportions of EDA domain in adult human hematopoiesis. Here we performed a study which lacks both EDA and EDB segments, and cellular fibronectin, a form found mainly as fibrils in the ECM and secreted by activated fibroblasts that contains variable proportions of EDA segments, EDB segments, or both. Recently we demonstrated the expression of the Extra Domain A (EDA) fibronectin isoform in human megakaryocytes. However, little information exists about the expression of fibronectin containing the EDA domain in adult human hematopoiesis. Here we performed a comprehensive in vivo study of the hematopoietic stem cell (HSC) compartment in adult mice constitutively including or excluding the EDA domain exon and show that the production of mature blood cells, lineage distribution within hematopoietic organs, frequencies of the most primitive HSC populations are comparable to those of wild-type littermate controls. Finally, EDA inclusion/exclusion result in equal megakaryocytes bone marrow content, ploidy and platelet release in vitro as well as in vivo after lipopolysaccharide-induced thrombocytopenia. Therefore and its presence expressed by hematopoietic cells, EDA fibronectin is not a critical regulator of steady state or stressed hematopoiesis.


N-ACETYLCSYSTEINE INCREASES PROTEOGLYCAN SULFATION IN A MOUSE MODEL OF DIASTROPHIC DYSPLASIA

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Diastrophic Dysplasia (DTD) is a severe recessively inherited chondrodysplasia caused by mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene, leading to impaired uptake and intracellular depletion of sulfate. DTD patients show growth retardation, skeletal dysplasia, joint contractures and reduced viability. Functional impairment in the sulfate transporter causing low levels of intracellular sulfate determine glycosaminoglycan (GAG) undersulfation in newly synthesized cartilage proteoglycans (PGs).

Previous data have pointed out that sulfur-containing amino acids could have a potential role as intracellular sulfate sources when extracellular sulfate concentration is low or when its transport is impaired such as in DTD. On the basis of this observation we considered N-acetyl-L-cysteine (NAC) as a potential sulfation source. In this study we used our mouse model of diastrophic dysplasia (ddt mouse) to evaluate the contribution of NAC to cartilage PG sulfation in the fetal period. Since cartilage and bone growth and remodelling are processes that occur also in the early stages of development we administrate in the drinking water a solution 30 g/L NAC to pregnant mice from the first day of pregnancy to delivery. Then wild-type and mutant animals were sacrificed at P1 and cartilage PG sulfation was evaluated. GAGs were recovered from cartilage of the femoral head by digestion with papain and subsequent precipitation with cetylpyridinium chloride. Purified GAGs were digested with both chondroitinase ABC and ACII and disaccharide sulfation was evaluated by HPLC. A significative increase in PG sulfation was observed in mutant mice treated with NAC compared to the ones treated with a placebo. The amount of mono-sulfated disaccharides (ΔDi-4S and ΔDi-6S) relative to the total amount of disaccharides (ΔDi-0S, ΔDi-4S and ΔDi-6S) observed in mutant mice treated with NAC compared to the normal value 83%). Furthermore the skeleton of newborn treated mice was about 78% vs 63% in untreated dtd mice (normal value 83%).

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These data confirm that sulphur-containing amino acids such as NAC can be a source of sulfation for PG in vivo and suggest potential therapies of DTD with thiol compounds.

Acknowledgment. Work supported by PRIN-MIUR (grant no. 20094C2H2M).
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