anisms of various systems cross. Disturbance of "integration link" at the collagen metabolism level is shown by an "experimental osteochondrosis" model (distrophycally destructive process in the spine proved hystologically). It was provoked by the separation of an intervertebral disk from the vertebral body by two vertebral segments after a lumbar spine operation (white male rats of 12 month age). The tissues of intervertebral disks, bodies of vertebrae, femoral bones, myochardium and aorta were taken for the analysis. The 14C-proline radioactive tracer was injected intraperitoneally 24 h before the animal decapitation. The decrease in the amount of the total proline has been found in all the observed tissues, the intensity the 14C-proline absorption has increased in all the tissues except the vascular one, but the hydroxyproline concentration has changed selectively: it increased in femoral and vertebral tissues and decreased in heart, vessels, intervertebral disks. It is possible to suppose that in the osteal tissues it is directed to the collagen synthesis. At the same time it is used for other purposes in the intervertebral disks, cardial and vascular tissues, for example, for energetical ones. A competition for the proline is possible between the tissues. It may be basis for combination of various organ pathology.

D1-027P
Effect of vasoactive peptides on adhesion and chemotaxis elicited by extracellular matrix protein sequences

L. Kun[1,3], H. Süli-Vargha[2], N. Mihala[2], M. Tóth[3] and L. Kõhidai[1]
[1]*Chemotaxis Research Group, Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary,* [2]*Reserach Group of Peptide Chemistry, Eötvös Loránd University of Sciences, Budapest, Hungary,* [3]*First Department of Internal Medicine, Semmelweis University, Budapest, Hungary.*
E-mail: kunlid@dgci.sote.hu

Vasoactive peptides are considered to be regulatory factors in physiological disorders. The interaction of cells with extracelluar matrix (ECM) is important in cell physiological processes. Constitutive sequence RGD of ECM proteins is recognized by the integrins. Obtustatin is α1β1-specific disintegrin, have the KTS integrin-binding motif. A non-integrin receptor for ECM components is elastin-binding protein (EBP), a specific binding site of VGVAPG motives of the elastin.
Objectives: To investigate (i) the capability of RGD, KTS and VGVAPG peptides to induce *in vitro* cell adhesion and chemotaxis; (ii) the relation of adhesion and chemotaxis in cells being on distinct levels of dedifferentiation; (iii) the influence of vasoactive peptides: angiotensin-II (ATII), endothelin-1 (ET-1), apelin-13 (Ap13), on the above-mentioned parameters.
Methods: Applied model-cells: THP-1 and J-774 monocytes, and MRC-5 fibroblasts. The chemotactic ability was determined in NeuroProbe® chamber. The cell adhesion assays were done with peptide-coated immunoplates.
Results: (i) All three ECM peptides elicit a concentration-dependent, adhesion in J-774 and MRC-5 cells, while adhesion of the most dedifferentiated THP-1 monocytes was induced slightly by the specific KTS peptide. (ii) VGVAPG and RGD peptides, have a strong chemoattractant effect on MRC-5 and J-774, at higher concentrations (10^{-6}M), while THP-1 cells was sensitive to the KTS peptide. (iii) Cell pre-treatments with vasoactive peptides perturb their responsiveness with diverse, vasoactive peptide-specific outcome.
Conclusion: The investigated peptides have ligand-specific effects in different cell lines. The distinct influence of the vasoactive peptides suggests their paracrine regulatory role on cell migration.

D1-028P
Regulation of MMP-13 by nitric oxide and association with caveolin-1

E. Lopez[1,2], T. Rodriguez[1,2], J. M. Lopez[3], I. Rodriguez[4], A. Alvarez[1], S. Lamas[1,2] and C. Zaragoza[1,2]
[1]*Vascular Biology, Departament of Structure and Funtions of proteins, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain,* [2]*Vascular Biology, Department of Strucutre abd Funtions of proteins, Centro de Investigaciones Biologicas, Madrid, Spain,* [3]*Vacular Pathology, Departament of de Physiology and Pharmacology, University of Salamanca, Salamanca, Spain,* [4]*Vascular Biology, Department of Bioquimica y Biología Molecular, University Complutense, Madrid, Spain. E-mail: elopez@cnic.es*

Matrix metalloproteinase (MMPs) are implicated in matrix remodeling during proliferative, inflammatory and angiogenic process including wound healing, whereas VEGF is a critical cytokine involved in angiogenesis, and nitric oxide (NO) is a downstream effector. We have shown that NO induces MMP-13 expression and activity in bovine and mouse aortic endothelial cells. We have demonstrated that aortic endothelial cells from eNOS null mice present delayed migration and a significant decrease of MMP-13 expression. We also demonstrated that MMP-13 was localized to caveolae, forming a complex with caveolin-1. Caveolins are structural proteins used by cells to form caveolae, involved in normal signal transduction pathways and in the pathogenesis of several pathological entities. In an effort to determine the precise mechanism by which MMP-13 interacts with caveolin-1, we identified the caveolin-1 Scaffolding domain as a docking region to which MMP-13 is bound, as detected by incubation with a synthetic peptide comprising the caveolin-1 scaffolding domain. Stimulation with NO disrupted this complex and significantly increased extracellular MMP-13 abundance, leading to collagen breakdown. Taken together these results indicate that MMP-13 mediates nitric oxide activation of endothelial cell migration.

D1-029P
Decorin transfection in breast cancer cells induces proteomic modulation and downregulation of matrix proteases

S. Minafra, G. Di Cara, **L. Minafra** and S. Feo
Laboratorio di Genomica e Proteomica, Dipartimento di Oncologia Sperimentale e Applicazioni Cliniche, Centro di Oncobiologia Sperimentale, Università di Palermo, Palermo, Italy.
E-mail: minafra@unipa.it

The progression of cancer is associated with multiple gene alterations and/or defective gene expressions. During the invasive growth neoplastic cells enter in dynamic contact with several components of the extracellular matrix that may influence gene expression and induce phenotypic modulation of neoplastic cells. Among these environmental factors, decorin, a representative member of the small leucine-rich proteoglycan family, occupies a central role because of its ability to interact with collagens and cellular receptors and to modulate biological activities. To test the effects of ectopic decorin expression in neoplastic cells we performed a comparative study of proteome and matrix proteases of decorin-transfected 8701-BC clones vs. the parental cell line, applying 2D-IPG, zymography, Western blot and RT-PCR methodologies. Our preliminary results showed that the protein complement expressed by cells following transfection undergoes significant modifications. Protein modulation involves some cytoskeletal proteins, metabolic enzymes and chaperonins. Cell morphology assays show remarkable cell surface modifications of

transfected clones. Since cytoskeleton, besides its role in maintaining cell polarity, is also involved in signal transduction, its modification in the transfected clones is probably associated with complex responses induced by ectopic decorin. In addition, transfected clones display a significant reduction of the levels of matrix proteases released into the media culture, which correlates with a downregulation of the transcription of the corresponding genes. These results provide additional insights into the reported effect of decorin in neoplastic cell behaviour.

Acknowledgment: Work supported by MIUR cofin to S.M. 2002.

D1-030P
Distribution of hyaluronan and hyaluronan-associated proteins in the spinal cord of chicken embryos

Z. Mészár[2], K. Matesz[1,2], Z. M. Szigethy[1,2], G. Veress[1,2], G. Székely[1,2], S. Felszeghy[1,2] and L. Módis[1,2]
[1]*Department of Anatomy Histology and Embryology, University of Debrecen Medical and Health Science Center, Debrecen, Hungary,* [2]*Tissue and Neuroscience Research Group, Subsidized Research Unit of The Academy of Sciences, Budapest, Hungary.*
E-mail: meszarz@chondron.anat.dote.hu

Hyaluronan is a peculiar non-sulfated glycosaminoglycan that is generally present in the extracellular matrix (ECM). The interaction of HA with HA-binding ECM molecules or CD44 and RHAMM cell surface receptors regulates many aspects of cell behavior including cell migration, differentiation and cell adhesion to another cell or ECM. Based on our studies, we speculate whether the HA acts as an autocrine or paracrine regulator through hyaluronan receptors (CD44 and RHAMM) or it is involved in a different signaling pathway. By using a specific HA binding probe derived from aggrecan we found strong HA signal in the intermediate zone in the cross-sections of chicken embryos from the age of 23 stages according to Hamburger and Hamilton (HH) while the other part of the spinal cord showed a moderate (marginal zone) or loss of (germinative zone, floor plate) HA signal. We could not find any CD44 expression in the spinal cord of the chicken embryos until they reach the HH36 stage. By using RT-PCR we have demonstrated that HA found in the spinal cord of chicken embryos is produced by hyaluronan synthase 2 (has2). HA reaction in the intermediate zone in the developing spinal cord of chicken embryos may indicate a permissive role of the HA molecule during the early stage of neuronal development. It is known that HA is produced by has2 requires upstream Rac1 small GTPase which is thought to have an effect on actin polymerization during lamellipodia formation through CD44 or RHAMM receptor. In our experimental model, however the involvement of CD44 can be neglected in this signaling pathway.

D1-031P
Abnormalities of syndecan-1 expression in precancerous and malignant lesions of the oral cavity and uterine cervix

M. Máthé[1], Z. Suba[2], T. Füle[1], P. Tátrai[1], Z. Németh[2], Z. Papp[3] and I. Kovalszky[1]
[1]*1st Institute of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary,* [2]*Department of Oral and Maxillofacial Surgery, Semmelweis University, Budapest, Hungary,* [3]*1st Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, Hungary. E-mail: mathem@korb1.sote.hu*

Syndecan-1, a transmembrane proteoglycan may exert antiproliferative effects, but may also promote cell growth by binding various growth factors. Malignant epithelial cells often downregulate their own syndecan-1 production, whereas they are capable of inducing an aberrant syndecan-1 expression in stromal fibroid cells. Immunohistochemical analysis performed on 35 oral leukoplakias, 51 invasive oral squamous cell cancers, and 39 cervical carcinomas revealed one or both of the above alterations concerning syndecan-1 expression. A decrease in syndecan-1 expression compared with normal epithelium could occasionally be detected as early as in leukoplakias, representing pre-malignant oral lesions. Syndecan-1 expression of tumor cells was decreased or even completely lost in 45 of 51 oral carcinomas and 37 of 39 cervical carcinomas. Furthermore, tumor-induced stromal syndecan-1 immunoreaction appeared in 19 of 51 oral tumors. In the case of oral cancers, but not of the cervical carcinomas, the probability of postoperative progression showed some dependence on the degree of decrease in tumor cell syndecan-1 levels; still the correlation was not statistically significant. Based on recurrence and overall survival data, stromal syndecan-1 expression in primary oral cancers appears to be a more reliable factor of adverse prognosis; however, the question whether the presence and extent of stromal syndecan-1 expression can be considered real risk factors of postoperative progression in oral malignancies requires further investigation.

D1-032P
Angiotensin II induces a tyrosine kinase-dependent increase in metalloproteinase activity in endothelial cells

M. Montiel[1], E. Perez de la Blanca[1], J. Quesada[1], I. Gonzalez[1] and E. Jiménez[1]
[1]*Bioquímica, Biologia Molecular y Química Orgánica, University of Málaga, Malaga, Spain,* [2]*Hospital Carlos Haya, Malaga, Spain. E-mail: mmontiel@uma.es*

Matrix metalloproteinase 2 (MMP 2) comprise a subfamily of metalloproteinases (MMPs) capable of digesting basement membrane proteins. High expression of MMPs has been reported in various pathologic conditions associated with angiogenesis and tumor invasion. Angiotensin II (Ang II), a bioactive peptide of renin-angiotensin system, regulates numerous physiologic responses, such as salt and water balance, blood pressure, vascular tone, and vascular remodeling, and it is also involved in the pathogenesis of a number of cardiovascular diseases. In the present study, we demonstrated that Ang II provokes a dose-dependent increase in MMP 2 activity in lysates and conditioned media of human umbilical vein endothelial cells (HUVEC). Pretreatment of cells with 10 μM PP2, a selective inhibitor of Src family tyrosine kinase, or 10 μM U73122, a specific inhibitor of phospholipase C (PLC), markedly decreased Ang II-induced MMP 2 activity. Nevertheless, pretreatment of HUVEC with 0.5 μM wortmannin, a selective inhibitor of phosphoinositide 3-kinase (PI3K), does not modify Ang II-induced MMP 2 activity. These results seem to indicate that Ang II modulates the synthesis and secretion of MMP 2 from endothelial cells through a PLC and Src tyrosine kinase-dependent pathway, and suggest that PI3K is not involved in Ang II-induced MMP 2 regulation.

D1-033P
Structural determinant on self-assembly of modeled human elastin polypeptides

M. Miao[1], C. M. Bellingham[1], R. Stahl[1], E. Sitarz[1], S. Lee[1], J. T. Cirulis[2] and F. W. Keeley[1,2]
[1]*Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada,* [2]*Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada. E-mail: m.miao@utoronto.ca*

Elastin is an extracellular matrix protein found in large blood vessels, lung, ligaments, and skin, imparting the physical properties