

0842

SPONTANEOUS TRANSFORMATION OF LYMPH NODE AND BONE MARROW STROMAL CELLS FROM CANCER PATIENTSL. Kideryova,¹ R. Pytlik,¹ T. Soukup,² J. Karbanova,² L. Kucerova,³ S. Micuda,² H. Ruckerova,² P. Cervinkova,¹ M. Trneny,¹ J. Mokry,² P. Klener¹¹1st Medical Faculty, PRAHA ², Czech Republic; ³Medical Faculty, HRADEC KRALOVE, Czech Republic; ³University Hospital, HRADEC KRALOVE, Czech Republic

Background. Until recently, human cells were regarded resistant to spontaneous *in vitro* transformation. Last year, two papers in Cancer Research and Cytotherapy reported that diploid mesenchymal stem cells (MSCs) can convert to malignant phenotype *in vitro* in presence of high concentration of fetal calf serum. AIMS. We have obtained our first transformed stromal cell line in 2003. Since then, we have been studying conditions necessary for *spontaneous in vitro* transformation and *in vitro* and *in vivo* properties of transformed stromal cells. **Methods.** Lymph node stromal cells were obtained from patients undergoing diagnostic or curative surgical procedure for lymph node (4 patients) or epithelial cancer (3 patients). Bone marrow MSCs were obtained from patients undergoing diagnostic or staging bone marrow biopsy. After tissue disaggregation, cells were centrifuged on Ficoll gradient and mononuclear cells were allowed to adhere to tissue culture plastic. Adherent cells were grown in α -MEM with 10% fetal calf serum (FCS) or in α -MEM with 2% FCS supplemented with dexamethason, ascorbic acid, EGF and PDGF-BB. Surface, cytoplasmatic and nuclear antigens were studied by flow cytometry and immunofluorescence. Cytogenetic analysis was performed after standard G-banding. Transformed cells were injected subcutaneously or intraperitoneally into sublethally irradiated NOD/LtSzRag1null mice and tumors were examined histologically. **Results.** We have obtained transformed stromal cells from all seven lymph nodes grown in α -MEM with 10% FCS. Transformation occurred very quickly, during initial expansion in one case and from 5th to 10th passage in other cases. Only two transformed cell lines were obtained from more than twenty bone marrow aspirates and in both cases, the transformation occurred during 2nd passage. Before transformation, cell cultures did not undergo neither senescent nor crisis phases and normal cells were very quickly overgrown by morphologically abnormal cells with average doubling time of 38 hours. Immunophenotypically, these cells resembled MSCs and were CD90+, CD166+, CD34-, CD45-, cytokeratin- and CD117+. They were also positive for telomerase, grew without contact inhibition and were unable to differentiate into osteoblasts or adipocytes. Transformed cells were hyperdiploid to hypertetraploid (49-115 chromosomes), with nonrandom pattern of chromosomal gains and losses. When administered subcutaneously into immunodeficient animals, these cells produced locally invasive sarcomas and in several cases, visceral metastases were found after intraperitoneal implantation. On the other hand, cells from the same samples grown in MAPC medium with 2% FCS only retained their usual spindle-shaped morphology, contact inhibition, diploid karyotype and ability to differentiate into specialized cells. **Conclusions.** Stromal cells from cancer patients lymph nodes were prone to quick malignant transformation, while mesenchymal stem cells from bone marrow were much more resistant. For transformation, growth medium with 10% FCS was required in both cell types. After transformation, all the cell lines had very similar phenotype, karyotype and clinical behaviour. Whether the easy *in vitro* transformation is an inherent feature of lymph node stromal cells or reflects the wide-spread genomic instability of cancer patients remains to be established.

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0843

DOWN REGULATION OF ACTIVIN A BY LYMPHOMA IN THE BONE MARROW: A POSSIBLE MECHANISM OF BONE MARROW INVOLVEMENTM.Z. Haran,¹ R. Parmawasan,² E. Feldberg,¹ M. Fogel,¹ A. Berrebi,¹ M. Shtalrid,¹ L. Shvidel,¹ A. Duek,¹ A. Nagler,³ M. Leiba,³ D. Zippori²¹Kaplan Medical Center, Rehovot, Israel; ²Weizmann Institute of Science, Rehovot, Israel; ³Chaim Sheba Medical Center, Ramat Gan, Israel

Backgrounds. Increasing evidence points to the crucial role of the interaction of malignant lymphoma cells with their microenvironment that consists of mesenchymal, endothelial and other cells. The tumor stroma may either promote, or alternatively inhibit, tumor dissemination.

Our previous investigations indicated that the stromal cytokine activin A induces apoptotic death of myeloma cells due to its antagonism with the growth promoting effect of interleukin-6. Activin A belongs to the transforming growth factor (TGF) β superfamily. It has initially been studied in the reproductive system, but has also been implicated in the regulation of hemopoiesis; it is an erythroid differentiation factor and is expressed within the bone marrow (BM) microenvironment. Activin A functions are tightly regulated by the competitive inhibitor inhibin A and the binding inhibitors, follistatins. We previously showed that abundance of activin A was restrictive for B-cell production *in vitro* and that within human nasal polyps activin A expression was widespread, but it was absent from foci of B lineage cells. We were therefore interested to find out whether activin A plays a role in the occurrence of BM involvement.

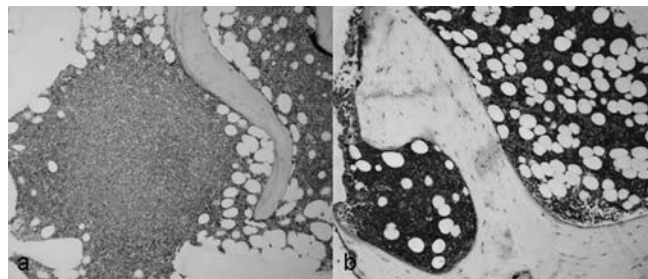


Figure 1. Low power view of activin A in the bone marrow.

Methods. The patient population consisted of all consecutive patients diagnosed with lymphoma between the years 2000-2004. In accordance with the IRB of our hospital, paraffin embedded sections were prepared and immunohistochemical staining was performed using an antibody to activin A. The slides were reviewed by team of 5 investigators and graded separately. We analyzed 17 patients with lymphoma and 3 patients without lymphoma served as controls. Results. Out of 17 lymphoma cases, 10 patients showed BM involvement while 7 patients were without BM involvement. In the former group the level of activin A was significantly decreased in the area surrounding the lymphoid infiltrate (Figure 1A). This was seen uniformly in all the patients except for one, regardless of the original histology of the tumor (follicular or diffuse). The level of activin A in the rest of the BM was similar to the level seen in specimens of reactive BM. In all 7 patients who had no BM involvement we found a diffuse staining for activin A (Figure 1B) (similar to what we saw in patients with reactive BM). **Conclusions.** Lymphoid cells have the ability to migrate to the bone marrow. It is interesting therefore that only some of the patients with malignant lymphomas have BM involvement. This could stem from a difference in the migratory abilities of the lymphoid cells, which is unlikely, or from a difference in their ability to home and flourish in the BM microenvironment. We demonstrated that activin A is significantly down-regulated in the vicinity of the 'metastatic' lymphoma, as opposed to what occurs in normal inflammatory BM. This suggests that an interaction between the lymphoma cell and the BM microenvironment leads to down-regulation of activin A expression and possibly promotes the survival of the lymphoid cells.

0844

EVALUATION OF THE EXPRESSION OF ANGIOGENIC CYTOKINES AND THEIR RECEPTORS IN AUTOIMMUNE MYELOFIBROSISR. Rizzi,¹ G. Guaragna,² E. Rinaldi,² A. Napoli,³ D. Pastore,² G. Specchia,² R. Ricco,³ V. Liso²¹Bari University Medical School, Bari, Italy; ²Department of Hematology, Bari, Italy; ³Department of Pathology, Bari, Italy

Background. Autoimmune myelofibrosis (AM) is an emerging clinicopathological entity, resulting in various degrees of isolated or combined chronic peripheral blood cytopenias. It is defined by a pattern including: increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration in bone marrow biopsies; absence of significant teardrop poikilocytosis and leukoerythroblastosis on peripheral blood smears; normal sized spleen; positive autoimmune serology, possibly fulfilling the classification criteria of an autoimmune disease. It has to be distinguished from different conditions associated with myelofibrosis; among these, the most relevant differential diagnosis is with chronic idiopathic myelofibrosis (CIM), particularly when disclosing autoimmune clinical and/or laboratory features. **Aims.** We purposed to assess the bone marrow stromal changes in AM with particular regard to the