Dov Zipori

Michal Cohen
Smadar Lapter
Ayelet Laronne
Reshmi Parameswaran
Varda Segal
Nir Shani
Yaron Shav-Tal

Regulation of hemopoiesis by the mesenchymal stroma: Role of cytokines and nuclear proteins

Department of Molecular Cell Biology

Tel. 972 8 934 2484 **Fax.** 972 8 934 4125
E-mail: dov.zipori@weizmann.ac.il

Blood forming tissues are organized in well defined microenvironments composed of hemopoietic cells and a stroma of mesenchyme and endothelium. Hemopoietic cells mature into various lineages, all derived from a small population of pluripotent stem cells residing in the bone marrow. Whereas much is known about the mode of induction of stem cell differentiation, insufficient information is available to explain the process of stem cell renewal which is crucial for the longevity of the hemopoietic system. Little is known as to how inhibition of hemopoietic processes occurs, and which are the molecules in the blood forming tissues that signal organization into discrete patterns. We derived a series of mesenchymal and endothelial cell lines from the bone marrow and use these to identify and isolate molecules involved in the organization of the bone marrow into discrete cellular microenvironments. This process seems to be regulated, at least in part, by members of the transfroming growth factor (TGF)β superfamily. Our study is focused on one member, activin A. *In vivo* and in vitro studies indicated that activin A is a negative regulator of B cell lymphopoeisis. Based on this study we formulated a theory termed 'the restrictin mode of cell organization' that explains tissue and organ structure on grounds of activity of negative regulators (Fig. 1).

The biological consequences of activin A action are mediated through direct control of the cell cycle but also through interference with the signaling cascade evoked by interleukin-6. One objective of our research is to identify, within the IL-6 signaling pathway, molecules which are targets for activin A. We studied IL-6 induced secretion of the acute phase protein haptoglobin by hepatoma cells. Overexpression of the C/EBPβ gene, a downstream effector in the IL-6 pathway, activated transcription from the haptoglobin promoter. This was abolished by either a constitutively active form of activin A type IB receptor (CAactRIB), or by a combination of Smad3 and Smad4. The transcription co-activator p300 partially overcame the suppressive effect of Smads. Electro-mobility shift assays indicated that C/EBPβ binding to Hp promoter DNA was reduced by over-expression of CAactRIB and Smad4. We thus show that Smad proteins operate as transcription inhibitors on target genes of the IL-6 induced pathway. This effect apparently involves interference with interactions among components of the transcription activation complex and with DNA binding.

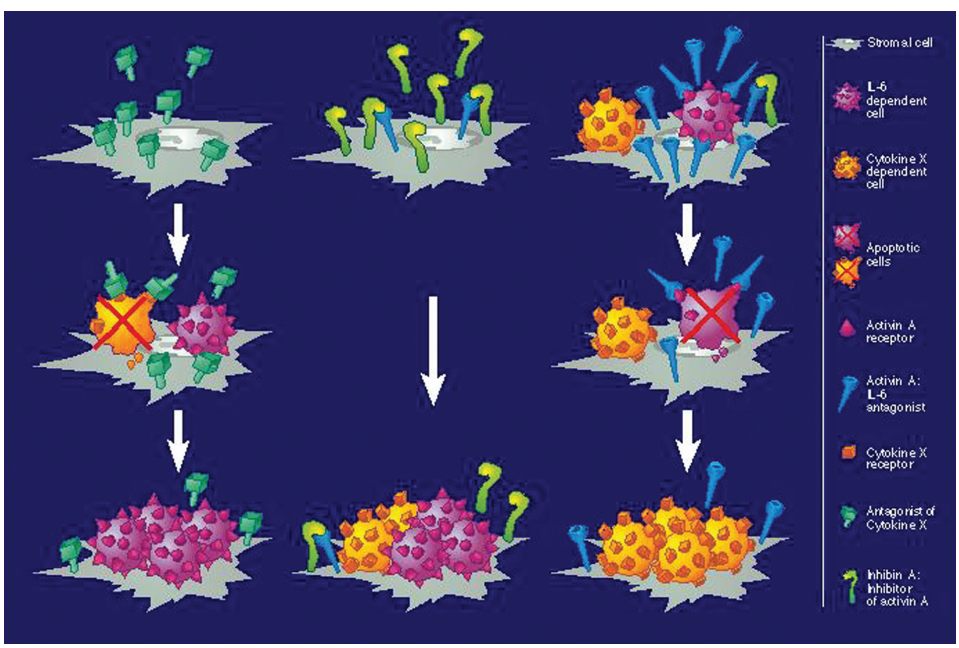

Fig. 1 *The restrictin mode of cell organization within tissues. Particular stromal cells express activin A (an IL-6 antagonist) preventing accumulation of IL-6-dependent cells at their close vicinity (right), unless these stromal cells express an excess of activin-inhibitors (middle). This specific case can be extended to any cytokine/antagonist combination (left).*

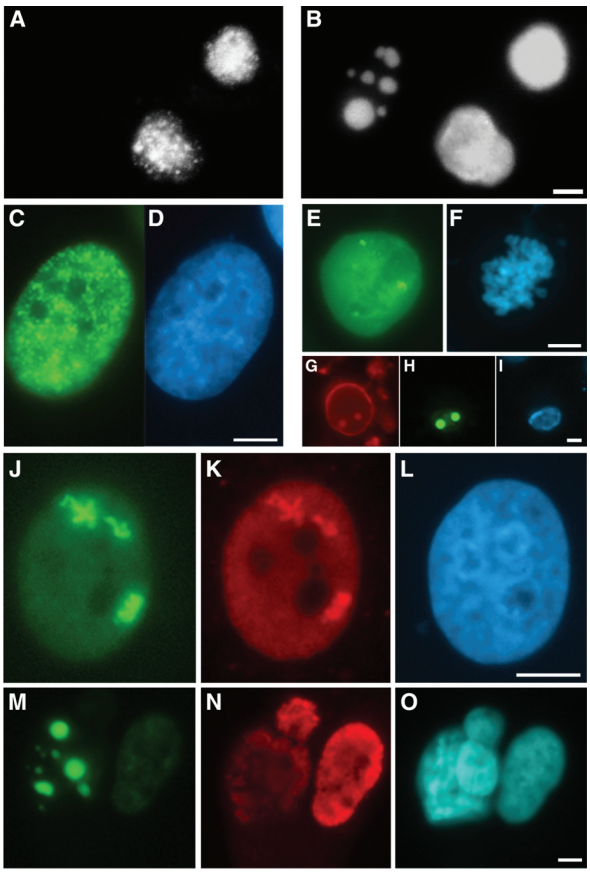

Fig. 2 *A) PSF localization. B) Apoptotic nucleus (DNA). C) GFP-PSF transfected cells, E) metaphase. G) Annexin-V-positive apoptotic cells. H) GFP-PSF localizes in aggregates. J) GFP-PSF in G1/S-blocked cells, K) double stained with anti-PSF. M) Apoptotic cells from cultures transfected with GFP-PSF, N) stained with anti-PSF and with O) DNA stain.*

The hemopoietic system presents a complex hierarchy of differentiation stages and lineages. A variety of nuclear proteins involved in hemopoietic differentiation have been identified. Many of the participants in the network are still missing. We study the role of one such nuclear protein, a pre-mRNA splicing factor, PSF, in the regulation of major cellular processes including differentiation, mitosis and apoptosis (Fig. 2). We previously described an immature myeloid cell nuclear antigen which was absent in mature granulocytes, implying a possible role for the antigen in differentiation. This antigen, detected in Western blots as a 49 kD protein, was purified to homogeneity and found to be a proteolytic product of the 100 kD pre-mRNA splicing factor PSF. The extreme sensitivity of PSF to the myeloid protease was not the cause of lack of detection of PSF in intact mature myeloid cells since inhibition of transcription caused reappearance of immunofluorescence detectable PSF. Two other cellular processes, mitosis and apoptosis, were characterized by a lack of PSF detectable by immunostaining whereas this protein was found intact and at normal levels by Western blotting. We showed that PSF is constitutively phosphorylated on serine and threonine residues and that myeloid differentiation, as well as cell cycle progression and apoptosis in different cell types involves hyper-phosphorylation. Utilizing GFP-PSF constructs we localized the region of hyper-phosphorylation to its N-terminus. The correlation between the phosphorylation state of PSF and its reactivity with antibodies suggests that the masking of the antigenic epitope involves binding of new protein partners during processes involving nuclear reshaping.

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