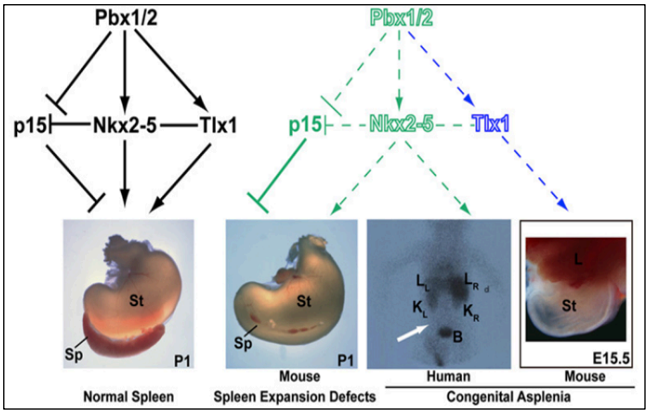
Research on Spleen Development in Animal Models – Selleri Laboratory, UCSF

In past years, the Selleri laboratory has devoted substantial efforts to gain insight into the genetic and transcriptional networks that control the development of a vertebrate organ with critical functions in blood filtering, immunity, and hematopoiesis, the spleen. In newborns and young children, both complete lack of the spleen (spleen agenesis or asplenia) and the presence of an atrophic spleen remnant (hyposplenia) result in a high risk for life-threatening bacterial infections by encapsulated bacteria, including pneumococcus pneumoniae and haemophilus influenzae. Among asplenic conditions, Isolated Congenital Asplenia (ICA) is the only known birth defect involving a lymphoid organ without additional developmental anomalies. ICA is an under-diagnosed primary immunodeficiency, that is often discovered only at autopsy, and is estimated to affect at least 1 in 600,000 births.

While immune functions of the spleen have been well characterized over the years, the specification, development, and growth of the splenic anlage (the primordium that will give rise to the organ), as well as the differentiation of the splenic stroma (the organ scaffold), remain poorly understood. We have studied spleen development using the mouse as a model and have established regulatory modules that are essential for spleen formation. We have reported that perturbations of these modules cause asplenia or hyposplenia in mice and humans. We have further identified novel genetic and regulatory cascades that control the successive stages of spleen genesis. In particular, Pbx genes are hierarchical regulators of genetic pathways active in the spleen. Mice with loss-of-function of Pbx genes exhibit asplenia or hyposplenia. However, the number of known genes required for spleen development remains as yet scarce and is related mainly to the murine spleen.



Genetic network underlying spleen development and its perturbations in mice and humans. Conditions of hyposplenia or asplenia in mice and humans. Spleen (Sp); Stomach (St). Patient's scintigraphy: white arrow indicates lack of spleen in the left abdomen. Bladder (B) ; Kidney (K); Lung (L) . L (left); R (right). Empty font indicates gene loss (Adapted from Koss *et al*. *Developmental Cell* 2012; Selleri, Zappavigna, and Ferretti. *Genes & Development*, In Press, 2019).

In collaboration with Dr. Jean Laurent Casanova's laboratory (Rockefeller University & HHMI), by using whole exome sequencing (WES; i.e. sequencing of all the genes that encode for proteins in the human genome), we discovered that NKX2-5, a gene-target of PBX1, is mutated in all individuals with ICA in a family from Congo, Africa. Subsequently, by WES, the Casanova laboratory identified germline mutations in the gene encoding RPSA (ribosomal protein SA; a component of the small subunit of the ribosome, which is a cellular organelle critical for protein synthesis) in a large proportion (55%) of ICA patients. To understand the spleen-restricted function of RPSA, Dr. Maurizio Risolino, an Instructor in the Selleri laboratory, has used genetic engineering to generate new mouse strains with *Rpsa* loss-of-function exclusively in splenic cells. Characterization of these new mouse models will enable a deeper understanding of the biological mechanisms that lead to ICA.

The search for new ICA-causing mutations in patients without identifiable mutations in *RPSA* (45%) and for *RPSA*-interacting genes, both ongoing in the laboratory of our long-standing collaborator Dr. Casanova, is essential towards improving the molecular diagnostics of this life-threatening primary immunodeficiency. Concomitantly, the mysterious pathogenesis of ICA calls for novel explorations into the mechanisms underlying asplenia or hyposplenia in tractable animal models with genetic mutations that are engineered and characterized in our laboratory. Our approach, based on the study of organismal models, will lead us to decipher the cellular and molecular causes underlying this devastating birth defect, which in turn will open the path towards novel therapeutic interventions.

Publications:

1. A Pbx1-dependent genetic and transcriptional network regulates spleen ontogeny.
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2. Hox cofactors in vertebrate development.
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3. Development and function of the mammalian spleen.
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   Developmental Cell 22(5):913-26, 2012.

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   Proceeding of the National Academy of Sciences USA 115(34):E8007-E8016, 2018.

8. "Building the Perfect Body": control of vertebrate morphogenesis by PBX-dependent regulatory networks.
   Selleri L, Zappavigna V, Ferretti E.
   Genes & Development, In Press (2019).