

Transcriptional Control in Inflammation and Cancer

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Activities 2007. A large body of epidemiological and experimental data have demonstrated a direct link between chronic inflammation and the development of several types of epithelial cancers worldwide, including hepatocarcinomas, colon carcinomas, gastric and prostate cancer. Cancer development in chronically inflamed tissues is often preceded by specific alterations of tissue differentiation, as in the case of intestinal metaplasia of gastric mucosa in patients with chronic gastritis, and Barrett's esophagus (squamous metaplasia) in patients with gastroesophageal reflux disease. Such alterations of tissue differentiation are entirely reversible as their mechanistic basis is represented by epigenetic (non DNA sequence-based) alterations of chromatin and a subsequent change in the accessibility and usage of the underlying genome. Experimental data suggest that polyclonal epigenetic changes associated with chronic inflammation may lay the ground for the acquisition of clonal genetic changes leading to tumor development.

Research activity in the laboratory is focused on two main research areas: understanding at the molecular level the control of inflammatory gene expression in order to identify possible new targets of anti-inflammatory drugs; and investigating the epigenetic mechanisms linking chronic inflammation to cancer development.

An outline of the research in the lab

1. Molecular control of inflammatory gene transcription.

Inflammation entails the induction (or repression) of hundreds of genes whose products contribute to different aspects of the response, like the recruitment of leukocytes, the induction of changes in vascular permeability, the activation of anti-bacterial responses, and in a subsequent step the induction of the repair response leading to reconstitution of tissue integrity.

The first objective of the lab is the mechanistic understanding of transcriptional regulation of inflammatory genes both in inflammatory cells (like macrophages) and in bystander cells exposed to an inflammatory environment. An in-depth

understanding of such mechanisms may provide the molecular basis for therapeutic targeting of subsets of NF- κ B-regulated transcriptional events (such as small-molecule-based drugs interfering with NF- κ B interaction with transcriptional coregulators). To achieve these objectives, standard biochemical approaches to transcription are integrated with bioinformatics, imaging and in vivo studies.

- **The NF- κ B family of transcription factors and the cancer connection.** Transcription of the vast majority of inflammatory genes relies on the activation of a family of transcription factors collectively known as NF- κ B. In mammals most cell types contain a collection of NF- κ B dimers composed by homo- and hetero-typic combinations of five transcription factors: p65/RelA, c-Rel, RelB, p50 and p52. These proteins contain a highly homologous Rel Homology Region (RHR) that mediates protein-DNA interactions and dimerization, as well as interactions with inhibitory proteins known as I κ Bs. Depending on the cell type and the differentiation status, the relative abundance of each dimer may vary, thus generating a high degree of complexity. The main regulatory switch in the NF- κ B system is cytoplasmic and consists in the release of NF- κ B from the I κ Bs. This activation step is mediated by the recently discovered I κ B kinase (IKK), which phosphorylates amino-terminal regulatory serines in the I κ Bs and targets them for proteasomal degradation, thus liberating the NF- κ Bs and allowing them to enter the nucleus. However, it is becoming increasingly clear that in addition to this required activation step, both NF- κ B recruitment to target genes and post-recruitment NF- κ B-induced transcriptional events are actively regulated. All NF- κ B dimers share the ability to bind a family of 9-11 nt DNA-binding sites collectively known as κ B sites and conventionally represented as G-5G-4G-3R-2N-1NoY+1Y+2C+3C+4 (R=purine, N= any nucleotide, Y= pyrimidine). In spite of similar DNA-binding profiles, NF- κ B dimers are not equivalent in terms of transcriptional activation properties since each one of them activates specific subsets of genes and has different potency at genes that are activated in a redundant fashion.

Understanding principles underlying transcriptional regulation

by NF- κ B proteins is an important scientific task with potential biomedical implications. NF- κ B is an essential regulator of several essential biological responses. First, it is required for the induction of the innate and adaptive immune response, regulating critical functions in essentially every cell type of the immune system. Second, it is both directly and indirectly connected to cancer development. Indirect connections arise from its essential role in the regulation of inflammation. NF- κ B activation in non-transformed cells of the tumor stroma, like fibroblasts, endothelial cells and macrophages contributes to tumor initiation and progression by mediating transcriptional induction of soluble mediators that amplify angiogenesis and neoplastic cell proliferation, and also affect progression to more advanced tumor stages. Direct connections depend on the role of NF- κ B as a switch in survival decisions in cancer cells: transcriptional activation of anti-apoptotic genes mediates such effects and modulates not only survival of the tumor but also its responsiveness to therapy.

Our lab is actively involved in unraveling basic mechanism linking NF- κ B to induction of inflammatory genes. This activity in the last years has been integrated with the use of advanced computational tools, mouse genetics and biophysics in order to obtain a detailed description of regulatory logic of the NF- κ B system.

- **Identifying transcriptional coregulators involved in inflammatory gene transcription.** Global inhibition of NF- κ B to block inflammation can be achieved nowadays by use of selective inhibitors of the pathway. However, such strategy is likely to cause major side effects due to the central role of NF- κ B in innate and acquired immunity as well as in the prevention of cell death in response to several stress stimuli. Our laboratory is actively involved in characterizing transcriptional coregulators controlling subsets of inflammatory genes in order to identify potential drug targets whose inhibition should impair the expression of a fraction of (pathogenic) inflammatory genes without completely disabling the whole response. We have already identified several enzymes that we are now extensively characterizing



in vitro and, in selected cases *in vivo*, in order to precisely determine the specific responses in which they are involved.

2. Inflammation and epigenetic control. The second main research area of the lab is focused on the epigenetic mechanisms that link chronic inflammation to cancer initiation and progression. We are analyzing how inflammatory stimuli modify activity or expression of epigenetic modifiers (including enzymes directly acting on chromatin) and how such changes impact on maintenance of genomic stability. We have already identified a number of enzymes whose expression is altered in response to inflammatory cues. One of these enzymes, *Jmjd3*, acts as a demethylase

specific for trimethylated lysine 27 ($H3K27me3$) in histone H3, a major constituent of chromatin. $H3K27me3$ is a chromatin modification essential to prevent unwanted activation of genes specific to alternative lineages. As such, this modification is essential to funnel differentiation of stem cells and precursors towards a given lineage. We are now using *in vivo* models in the mouse to define the role of this enzyme in differentiation abnormalities associated with sustained inflammation. Moreover, we are investigating the correlation between expression of *Jmjd3* and other chromatin modifiers in metaplasia associated with chronic inflammation of the upper gastrointestinal tract.