Abstract

The regulation of transcription of tens of thousands of genes in a vertebrate organism is an enormously complex phenomenon which entails the participation of thousands of various regulatory proteins. The largest functional category of these regulators is accounted for by sequence-specific DNA-binding proteins known as transcription factors. Proteins of the EGR and Myb families of transcription factors are long-studied regulators of a variety of physiological processes including cellular proliferation and differentiation. The structural and physical aspects of their function have been well characterized. Their cell-type specific participation in complex gene-regulatory networks, on the other hand, is still incompletely understood and represents a major challenge in the respective research areas.

Preliminary analysis of gene expression data from metastasizing PR9692 and non-metastasizing PR9692-E9 chicken sarcoma cell lines revealed that the transcription factor EGR1 is expressed at a higher level in metastasizing cells and can thus take part in the regulatory processes that underlie the differences between the two cell lines. Further investigation demonstrated that the introduction of exogenous EGR1 into PR9692-E9 cells restored their metastatic potential to a level indistinguishable from PR9692 cells. Microarray analysis of EGR1 reconstituted cells revealed the activation of genes that are crucial for actin cytoskeleton contractility (*MYL9*), integrin signaling (*RIAM*), filopodia formation (*MYO10*), the production of specific extracellular matrix components (*HAS2*, *COL6A1-3*) and other essential pro-metastatic abilities. Constitutive expression of EGR1 in PR9692-E9 cells also decreased the expression of numerous genes some of which have been identified in other studies as metastasis suppressors (*SFRP4*, *IRX1* and *CADM1*).

Fibrotic diseases are a group of pathologies with high incidence and mortality. Understanding the molecular mechanisms driving the onset, progression and possible resolution of fibrosis is a prerequisite to the development of successful therapies. The central role in fibrosis is played by myofibroblasts. The myofibroblast is a specific type of mesenchymal cell characterized by synthesis of the extracellular matrix (ECM), plus contractile and secretory activity. While these cells serve a beneficial function during physiological tissue wound healing, they can cause devastating fibrosis under pathological conditions. We have established that

chicken embryo dermal myofibroblasts (CEDM) represent a useful *ex vivo* model suitable for analyses of the myofibroblastic phenotype and regulation. We have also revealed that increasing the protein level of EGR4 in these cells induces a loss of myofibroblastic characteristics. We further analyzed this effect with respect to alterations in activity of signaling pathways and gene expression changes after EGR4 overexpression. As the major signaling system driving the differentiation of myofibroblasts is the TGF-β signaling pathway, we first identified TGF-β-regulated genes in CEDM cells using a specific chemical inhibitor of kinase activity of TGF-β receptor 1 and oligonucleotide microarrays. We revealed both genes previously reported in mammalian systems (e.g. SPON2, *ASPN*, *COMP*, *LUM*, *HAS2*, *IL6*, *CXCL12*, *VEGFA*, *NGF*) and novel TGF-β-dependent genes, among them *PGF*, *VEGFC*, *PTN*, *FAM180A*, *FIBIN*, *ZIC1*, *ADCY2*, *RET*, *HHIP*, *DNER* and *TMEM45A*. Our results on the long term inhibition of TGF-β signaling in CEDM cells suggests that TGF-β is primarily involved in the regulation of secretory activities including ECM production while contractile activity depends on additional regulatory signals. Next, we focused on the mechanisms that are employed by EGR4 to induce the observed dedifferentiation in CEDM cells. We found that sustained expression of EGR4 caused a strong inhibition of TGF-β signaling and also a suppression of the expression of genes encoding key components of the myofibroblast contractile apparatus. Microarray analysis identified a number of genes affected by EGR4 and among them a few candidates that may mediate some of the observed effects. Further experiments led to a hypothesis that *FOXG1*, *BAMBI*, *NAB1*, *NAB2* and *DUSP5* genes together form an EGR4 regulated network counteracting autocrine TGF-beta signaling.

Research on the role of Myb proteins in lineage commitment in hematopoiesis and melanocytogenesis was being performed in parallel with the EGR-related research. The development of blood cells proceeds from pluripotent stem cells through multipotent progenitors into mature elements belonging to at least 8 different lineages. The lineage choice process during which stem cells and progenitors commit to a particular lineage is regulated by a coordinated action of extracellular signals and transcription factors. Molecular mechanisms controlling commitment are largely unknown. We identified the ability of the transcription factor v-Myb^{AMV} to regulate the commitment of a common myeloid progenitor and progenitors restricted to the myeloid lineage and the role of its leucine zipper region (LZR) in these effects. We demonstrated that wild-type v-Myb^{AMV} with the intact LZR directs development of progenitors into the

macrophage lineage. Mutations in this region compromised commitment toward myeloid cells and caused v-Myb^{AMV} to also support the development of erythroid cells, thrombocytes, and granulocytes, similar to the c-Myb protein. We proposed that Myb LZR can function as a molecular switch, affecting expression of lineage-specifying transcription factors and directing the development of hematopoietic progenitors into either myeloid or erythroid lineages.

In addition to hematopoiesis, we also demonstrated cell fate-directing abilities of c-Myb and v-Myb^{AMV} proteins in avian neural crest (NC), where both proteins determine melanocytogenesis. The increased concentration of c-Myb induced progression into dendritic melanocytes and differentiation. The v-Myb^{AMV} oncogene converted essentially all NC cells into melanoblasts and caused their transformation. Both Myb proteins activated in NC cells the expression of the *c-kit* gene and SCF - c-Kit signaling – one of the essential pathways in melanocyte development. As *c-kit* was identified as a target of Myb proteins in hematopoietic cells in a previous study, our observations suggest that the c-Myb - c-Kit pathway represents a common regulatory scheme for both hematopoietic and NC-derived progenitors. Our work establishes a novel experimental model for studies of melanocytogenesis and melanocyte transformation.