

Chapter 6

The AP-2 α Transcription Factor Regulates Tumor Cell Migration and Apoptosis

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Abstract. AP-2 proteins are a family of developmentally-regulated transcription factors. They are encoded by five different genes (α , β , γ , δ , and ϵ) but they share a common structure. AP-2 plays relevant roles in growth, differentiation, and adhesion by controlling the transcription of specific genes. Evidence shows that the AP-2 genes are involved in tumorigenesis and for instance, they act as tumor suppressors in melanomas and mammary carcinomas. Here we investigated the function of the AP-2 α protein in cancer formation and progression focusing on apoptosis and migration. We introduced AP-2 α -specific siRNA (as oligos or in retroviruses) in HeLa or MCF-7 human tumor cells and obtained a pronounced down-modulation of AP-2 α mRNA and protein levels. In these cells, we observed a significant reduction of chemotherapy-induced apoptosis, migration, and motility and an increase in adhesion suggesting a major role of AP-2 α during cancer treatment and progression (migration and invasion). We have data suggesting that migration is, at least in part, regulated by secreted factors. By performing a whole genome microarray analysis of the tumor cells expressing AP-2 α siRNA, we identified several AP-2 α -regulated genes involved in apoptosis and migration such as FAST kinase, osteopontin, caspase 9, members of the TNF family, laminin alpha 1, collagen type XII, alpha 1, and adam.

1 Introduction

The AP-2 family of transcription factors consists of five closely related proteins of Mr 50,000, AP-2 α , β , γ , δ , and ϵ (Feng and Williams, 2003; Hilger-Eversheim et al., 2000; Zhao et al., 2001) encoded by distinct genes. These transcription factors can form homoor heterodimers via helix–span–helix motifs and transactivate their target genes by binding to GC-rich consensus sequences in the promoter regions (Williams and Tjian, 1991). AP-2 factors orchestrate a variety of cell processes including apoptosis, cell growth, cell adhesion, tissue differentiation and tumorigenesis

(Hilger-Eversheim et al., 2000). Generation and analysis of mice deficient in AP-2 α , β or γ have indicated important roles for AP-2 factors in the development of neural crest, urogenital, and epidermal tissues during embryogenesis (Moser et al., 1995; Moser et al., 1997; Schorle et al., 1996; Werling and Schorle, 2002). The crucial role of AP-2 genes in regulating gene expression is highlighted by the embryonic lethality of all these genetically modified mice. Some of the genes regulated by AP-2 transcription factors are p21WAF/CIP (Zeng et al., 1997), transforming growth factor- α (Wang et al., 1997), estrogen receptor α (McPherson et al., 1997), keratinocyte specific genes (Leask et al., 1991), c-KIT (Huang et al., 1998), adhesion molecules such as MCAM/MUC18 or integrins (Suyama et al., 2002), type IV collagenase/gelatinase/MMP-2, E-cadherin, VEGF (Nyormoi and Bar-Eli, 2003) and ERBB-2 (reviewed in Hilger-Eversheim et al., 2000). Several studies suggest that AP-2 plays a role in tumorigenesis acting as oncogene or tumor suppressor depending on the type of tumor (Hilger-Eversheim et al., 2000). Here AP-2 regulates gene expression directly by binding to the regulatory regions of some of the genes listed above or indirectly displaying functional protein–protein interactions with other transcription factors such as c-myc, pRB, and p53 (Hilger-Eversheim et al., 2000).

2 Results

2.1 *Downregulation of AP-2 α Expression in Tumor Epithelial Cells*

Many tumor cells and human epithelial tumors show downregulation of the AP-2 transcription factors (Hilger-Eversheim et al., 2000). Here we planned to downmodulate AP-2 α in tumor cells by using specific siRNA and study tumor formation and progression. HeLa (cervix adenocarcinoma) or MCF7 (breast adenocarcinoma) human cells were first transiently transfected either with generic nonsilencing (NS, Qiagen) or specific AP-2 α siRNA oligos (Qiagen, sequences in Figure 1) and analyzed for protein expression 48 h after transfection by western blot (Figure 2a). Alternatively, the cells were transduced with retroviruses and selected to obtain stable expression of pSUPERretro.puro-OLIGO2 or pSUPER retro.puro-OLIGO4. The empty pSUPERretro.puro was used as control. Protein expression was also analyzed by western blot (Figure 2b,c). A very significant reduction of AP-2 α expression was observed when the cells were either transfected with OLIGO2 or OLIGO4 siRNA oligos or transduced with pSUPERretro.puro-OLIGO2 or pSUPER retro.puro-OLIGO4 expression vectors compared with control cells (Figure 2).