passaged for a prolonged period of time in culture, undergoes a non-clonal change reflected in lower ERK activity, increased p38 activity and a dormant/quiescent phenotype in vivo (D-HEp3). When inoculated in vivo ~80% of D-HEp3 cells rapidly arrest in G_0/G_1 by day 6 after inoculation and remain dormant for several months. We also found that activated p38 establishes a negative feedback loop to ERK and that blocking of p38 by genetic or pharmacological inhibitors restores ERK activation and interrupts tumor dormancy in vivo. These studies implicated high p38 activity in the induction of dormancy in vivo. While p38 is known to induce growth arrest and/or apoptosis, there is also evidence indicating that in some instances, p38 signaling can promote cell survival. However, knowledge of the proximal targets of p38 that underlie the p38-dependent dormancy program, in particular the balance between cell proliferation and cell death, have not been identified. We now show that p38 regulates the activation of the endoplasmic reticulum (ER)-stress activated kinase PERK and expression of the ER chaperone BiP/Grp78. Regulation by p38 of these pathways allows dormant tumor cells to not only become dormant but also resist drug-toxicity. Increased activation of the eIF2α kinase PERK, results in upregulation of ATF4 and activation of GADD153 promoter. RNAi and dominant negative expression studies revealed that both BiP and PERK promote survival and drug-resistance of dormant cells and that BiP upregulation prevents Bax activation. Further, genetic experiments showed that activation of the PERK-eIF2α pathway is important for the maintenance of dormancy. We propose that stress-dependent activation of p38 that results in BiP upregulation and PERK activation may represent a novel growth arrest and survival mechanism that induces dormancy and protects dormant tumor cells from stress-insults such as chemotherapy.

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S9. ZNF306, A NOVEL ZINC FINGER TRANSCRIPTION FACTOR, DRIVES COLON CANCER PROGRESSION – AN ALTERNATE GENETIC PATHWAY IN TUMOR PROGRESSION?

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Colorectal cancer is the second leading cause of cancer deaths in western countries. Although inactivation of the *APC* and *p53* tumor suppressor genes coupled with Kirsten-*Ras* oncogene activation contribute to colon carcinogenesis, simultaneous mutation of these three genes is rare suggesting alternate genetic pathways leading to colon tumorigenesis/progression. To identify novel genes that contribute to colon cancer development/progression we "data-mined" genes aberrantly expressed in colorectal cancer using SAGE and UniGene Cluster Expression analysis and identified, a novel Scan domain-containing zinc finger protein (ZNF306) whose expression is elevated in colon cancer. RT-PCR analysis of resected colon cancers showed elevated ZNF306 mRNA levels in two thirds of tumors compared with paired adjacent non-malignant tissue. Stable expression of the cloned ZNF 306 cDNA in HCT 116 colon cancer cells yielded enhanced soft agar colony formation, anoikis resistance and resistance to 5-flurouracil when compared with cells bearing the empty vector. More importantly, orthotopic implantation of ZNF306-overexpressing HCT 116 cells yielded large tumors in 100% of the mice compared with vector only-expressing cells which produced smaller tumors with a lower penetrance (20% of mice). Conversely, transduction of two independent colon cancer cell lines with a-ZNF306 siRNA reduced mRNA levels, diminished colony size and attenuated cell proliferation. Further, in vivo delivery of neutral liposomal-encapsulated siRNA targeting ZNF306 reduced orthotopic growth of HCT116-ZNF306 cells. Flag-tagged expressed ZNF306 was nuclear-localized and since zinc finger-containing proteins recognize DNA site-specific sequences, we hypothesized that ZNF306 is a transcription factor. Cyclic amplification and selection of targets (CAST-ing) using a random oligonucleotide library identified the KRKGGGG nucleotide sequence as a putative DNA binding site. Expression profiling studies revealed several candidate downstream targets of ZNF306 including VEGF and integrin β4, implicated in angiogenesis and Ras/PI3-kinase signaling, respectively. Over-expression of these two putative targets was confirmed by RT-PCR. Additionally, increased CD31 (endothelial cells) immunoreactivity in the ZNF306-over-expressing orthotopic tumors indicated augmented angiogenesis. Both genes contained ZNF306 binding sites in their regulatory sequences and chromatin immunoprecipitation assays and EMSA, using an anti-ZNF306 antibody we generated, demonstrated binding of the ZNF306 protein to its recognition sequence (identified by CAST-ing) in the VEGF promoter indicating this gene to be a direct target of ZNF306. Immunohistochemistry employing the anti-ZNF306 antibody showed increased ZNF306 protein in colon cancer tissues compared with adjacent non-malignant mucosa. In conclusion, we have discovered a novel zinc finger protein, ZNF306 that contributes to colon cancer progression in part by elevating VEGF and integrin β4 expression. We propose that this gene product represents a key protein in an alternate genetic pathway leading to colon cancer progression.

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S10. SPECIFIC TRANSCRIPTIONAL REGULATORS OF THE u-PAR GENE – IN VIVO AND CLINICAL RELEVANCE, AND FIRST SUGGESTIONS FOR MOLECULAR TUMOR STAGING

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The urokinase-receptor (u-PAR) promotes the invasive and metastatic phenotype and has been shown to be associated with early relapse and poor prognosis in enumerous types of cancers. From our and other studies we know that high u-PAR gene expression in carcinoma cells is largely due to the transcriptional regulation of the gene. We have characterized two *cis*-elements (−152/−135, bound with an AP-2-like protein, Sp1, and Sp3; −190/−171, bound with AP-1-transcription factors) of the u-PAR promoter which are decisive for diverse means of u-PAR-gene expression in highly invasive colon cancer cells, among them being constitutive,