Six1 Overexpression Promotes Epithelial–mesenchymal Transition and Malignant Progression in Models of Cervical and Colon Cancer

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The overexpression of Six1, a member of the Six family of homeodomain transcription factors, has been found in various human cancers, and is associated with tumor progression and metastasis. We have previously determined that the expression of SIX1 mRNA increased during in vitro progression of human papillomavirus type 16 (HPV16)-immortalized human keratinocytes (HKc/HPV16) toward a differentiation-resistant (HKc/DR) phenotype. However, the mechanism(s) of how Six1 promotes HPV16-mediated transformation remain unknown. In this study, we explored the role of Six1 at early stages and late stages of HPV16-mediated transformation by overexpressing Six1 in HKc/HPV16 and HKc/DR. HKc/HPV16 and HKc/DR overexpressing Six1 exhibited a fibroblastic appearance and increased invasion compared to vector controls. Using a combination of microarray analysis, realtime PCR and Western blotting, we determined that overexpression of Six1 induced epithelial-mesenchymal transition (EMT) in HKc/HPV16 and HKc/DR. In addition, we observed alterations in the transforming growth factor-beta (TGF-β) receptors and activation of the non-Smad Mitogen-activated protein kinase (MAPK) pathway of TGF-β signaling in response to Six1 overexpression. Moreover, the overexpression of Six1 in HKc/HPV16 resulted in resistance to serum and calcium-induced differentiation; while Six1 overexpression in HKc/DR resulted in malignant conversion and increased the cancer stem cell (CSC)-like population. The activation of MAPK is linked to Six1-mediated resistance to calcium-induced differentiation in HKc/HPV16 and Six1-associated features of CSCs in HKc/DR.

We also used an orthotopic mouse model and a splenic injection metastasis model to investigate the role of Six1 overexpression in colorectal cancer (CRC) progression and metastasis. We found that overexpression of Six1 dramatically promoted CRC tumor growth and metastasis in vivo, increased features of cancer stem cells (CSCs), and stimulated angiogenesis by up-regulating the expression of vascular endothelial growth factor (VEGF). Moreover, we determined that Six1 overexpression resulted in the recruitment of tumor-associated macrophages (TAM), which further facilitates CRC tumor growth and metastasis. Furthermore, we determined that Six1 activated ERK and p38 MAPK signaling in MC38 CRC cells. In summary, our studies strongly suggest that Six1 overexpression promotes malignant progression in models of cervical and colon cancer, and MAPK activation may play a pivotal role in Six1-associated tumor progression.