

Abstract

Following treatment with a demethylating agent, 5 of 11 renal cell carcinoma (RCC) cell lines showed increased expression of hepatocyte growth factor (HGF) activator inhibitor type 2 (HAI-2/SPINT2/Bikunin), a Kunitz-type protease inhibitor that regulates HGF activity. As activating mutations in the MET proto-oncogene (the HGF receptor) cause familial RCC, we investigated whether HAI-2/SPINT2 might act as a RCC tumor suppressor gene. We found that transcriptional silencing of HAI-2 in RCC cell lines was associated with promoter region methylation and HAI-2/SPINT2 protein expression was down-regulated in 30% of sporadic RCC. Furthermore, methylation-specific PCR analysis revealed promoter region methylation in 30% (19 of 64) of clear cell RCC and 40% (15 of 38) of papillary RCC, whereas mutation analysis (in 39 RCC cell lines and primary tumors) revealed a missense substitution (P111S) in one RCC cell line. Restoration of HAI-2/SPINT2 expression in a RCC cell line reduced in vitro colony formation, but the P111S mutant had no significant effect. Increased cell motility associated with HAI-2/SPINT2 inactivation was abrogated by treatment with extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and phospholipase C-gamma inhibitors, but not by an inhibitor of atypical protein kinase C. These findings are consistent with frequent epigenetic inactivation of HAI-2/SPINT2, causing loss of RCC tumor suppressor activity and implicate abnormalities of the MET pathway in clear cell and papillary sporadic RCC. This information provides opportunities to develop novel targeted approaches to the treatment of RCC.