Carbonic anhydrase IX: target enzyme for diagnosis and therapy of renal cell carcinoma

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Renal cell carcinoma (RCC) accounts for about 3% of all solid malignancies and shows the highest aggressiveness among all urologic neoplasms. Cytokine based immunotherapy, INF-α and IL-2, has been used for the treatment of metastatic RCC (mRCC) since 1980s without long-term disease-free intervals. Substantial improvements in the outcomes have occurred with the development of therapies targeting the VEGF; anyway identifying therapeutic strategies that promote long-lasting remission of mRCC remains critically important (Weinstock et al., 2015).
The carbonic anhydrase (CA) family includes 16 catalytically active zinc metalloenzymes, that catalyze the reversible interconversion of carbon dioxide and water to bicarbonate and protons (Supuran CT., 2004). These isoforms mainly differ in their catalytic activity, tissue distribution and subcellular localization. CA IX and CA XII have been found to be overexpressed in a wide variety of human tumors, being involved in cancer aggressiveness and progression (Pastorekova et al., 2007). In particular, recent studies have revealed that CA IX is a downstream gene, activated following either hypoxia via HIF-1α (hypoxia inducible factor-1α) or VHL (Von Hippel-Lindau) inactivation. Carbonic anhydrase (CA) inhibitors have been proposed as a potential new class of antitumor agents; in fact, the selective inhibition of CA IX decreases tumour cell proliferation and induces ceramide-mediated apoptosis in human cancer cells (Cianchi et al., 2010). Recently, sulphonamide CA inhibitors targeting CA IX and CA XII entered in clinical trials for the treatment of advanced metastatic solid tumours. Moreover, CA IX is present at very low level in normal kidney tissue, suggesting that CA IX expression may be both a useful diagnostic marker and a therapeutic target to evaluated the effectiveness of therapy.
Tissue biopsies from patients with RCC were obtained from the Department of Experimental and Clinical Medicine, University of Florence (Italy). The protein expression of CA IX, VEGF and caspase-3 were evaluated by Western blot. The mRNA expression of CA IX was studied with RT-PCR. The activity of caspase-3 was determined and the concentration of VEGF in tissues was measured with a commercial ELISA kit. It was also assessed plasmatic concentration and activity of CA IX in healthy subjects and patients with benign kidney tumour and RCC.
The CA IX gene expression and protein concentration were significantly increased in tumour tissue compared to normal mucosa. As well as, VEGF concentration was higher in tumour tissue, while caspase-3 activity was significantly higher in the normal mucosa. These findings clearly demonstrate that VEGF is abundantly expressed in tumour tissue, and VEGF over-expression together with CA activity could be responsible for the observed significant inhibition of the signaling pathway of apoptosis, as evidenced by the reduced activity of caspase-3.
The concentration of CA IX in plasma of patients with RCC was significantly higher compared to healthy patients and patients with benign tumour. However, CA IX activity is increased in patients with RCC and with benign tumour, while it is reduced in plasma samples of healthy subjects.
These results show that CA IX is a useful marker for the RCC diagnosis and an interesting target for novel approaches in the anticancer therapy.

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