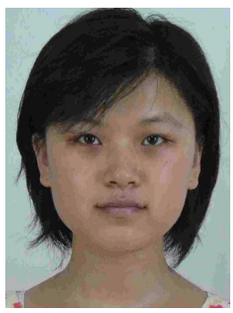
THE CO-OPERATIVE ROLES OF CD147 AND CD44 IN PROSTATE CANCER CHEMORESISTANCE AND METASTASIS



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Introduction: Prostate cancer (CaP) is the most common cancer in males. Although early stage CaP can be controlled using conventional therapies, multidrug resistance (MDR) and tumor metastasis remain the main causes of treatment failure and mortality in CaP patients.

Background: The relationship between tumor metastasis and multidrug resistance is not fully defined, although indirect evidence suggests a functional link between these processes. Extracellular matrix metalloproteinase inducer protein (CD147, EMMPRIN) is a multifunctional glycoprotein that can modify the tumor microenvironment by activating proteinases, inducing angiogenic factors in tumor and stromal cells, and regulating growth and survival of anchorage-independent tumor cells (micrometastases) and MDR. CD147 also regulates monocarboxylate transporters (MCTs), which are critical for lactate efflux in tumors interaction of CD147 with MCT1 or MCT4, within the endoplasmic reticulum, is necessary for MCT trafficking to the plasma membrane. CD44 is a multifunctional protein involved in cell adhesion, migration and drug resistance. CD44 is a key receptor for hyaluronan (HA), and the interaction of CD44 and HA co-regulates pathways important for cancer cell mobility, survival and MDR to chemotherapeutic drugs. Hence, it is plausible that interactions between CD147 and CD44 may play a role in the regulation of MDR and metastasis in CaP progression.

Purpose: This study aimed to: 1) to evaluate the expression of CD147, CD44s, CD44v3-10, MDR1, MCT1 and MCT4 in metastatic CaP cell lines and different grades of primary CaP tissues and to correlate it with clinicopathological parameters; 2) to evaluate the relationship of CD147 and CD44 with MDR1, MCT1 and MCT4 in CaP cell lines and primary CaP tissues; 3) to test docetaxel response and invasion in CaP cell lines with or without CD147 or CD44 expression; and 4) to examine whether CD147 knockdown modifies expression of CD44, MDR1 MCT1 and MCT4, and response of CaP cells to docetaxel and cancer invasion.

Methods: Expression of CD147, CD44s, CD44v3-10, MDR1, MCT1 and MCT4 was evaluated by peroxidase and fluorescence immunohistochemistry in tissue microarrays [TMAs -120 primary CaPs with different grades,

20 benign prostate hyperplasia (BPH) and 20 normal prostates], and in primary CaP. The expression of these markers was correlated with clinicopathological parameters. We also examined expression of CD147, CD44s and CD44v3-10, MDR1, MCT1 and MCT4 proteins in human metastatic CaP cell lines [PC-3M-4XD, PC-3M-luc-C6, PC-3, DU145, LNCaP-LN3, DuCaP), PC-3M-luc-K147-12 (CD147 knockdown), PC-3M-luc-NT-12 (control knockdown)] by immunofluorescence and confocal microscopy, as confirmed by immunoblotting. Chemosensitivity of CD147 knockdown and wild-type metastatic CaP cell lines to docetaxel was measured using MTT assays, and dose-response curves and sensitivity (IC50) were analysed (Graphpad Prism 4.0). CaP cell invasion was tested using Matrigel invasion assays.

Results: High levels and heterogeneous expression of CD147 CD44, MDR1, MCT1 and MCT4 were found in high grade (Gleason score >7) primary CaP. Moderate immunostaining was found in low grade CaP (Gleason score <7), whereas only weak staining was found in normal prostate for CD147 and CD44s. A number of high-grade CaP tumors showed heterogeneous co-localization of CD147, CD44s, CD44v3-7, MDR1, MCT1 and MCT4 on tumor and stromal cells. Over-expression of CD147, CD44v3-10, MDR1, and MCT4 in primary CaP tissues (TMAs) is correlated with CaP progression parameters including pre-treatment PSA level ($P<0.05$; Gleason score, $P< 0.05$; pathological stage, $P< 0.01$; nodal involvement, $P<0.05$ and surgical margin, $P<0.05$). Androgen nonresponsive metastatic cell lines (PC-3M-4XD, PC-3M-luc-C6, PC-3 and DU145) expressed high-level CD147, CD44, MDR1, MCT1 and MCT4, with co-localization of these markers in all cell lines. Androgen responsive DuCaP cells did not express CD147, CD44s, and MCT1, and showed weak CD44v3-10 and MCT4 expression. Androgen responsive, LNCaP-LN3 cells were CD147, MDR1 and CD44v3-10 (very low expression) and CD44s negative. PC-3M-luc-C6 cells (CD147++/CD44++) were more docetaxel-resistant (IC50 450nm) and more invasive compared to DuCaP cells (CD147-/CD44-) (IC50 10nM). CD147 and CD44 expression in metastatic CaP cells was positively related to docetaxel sensitivity (IC50) and invasive potential. PC-3M-luc-K147-12 cells (CD147 knockdown) showed reduced levels of CD44, MDR1, MCT1 and MCT4, and increased sensitivity to docetaxel. Invasiveness of CD147 knockdown cells was reduced compared to control cells.

Conclusions: We show, for the first time, co-expression of CD147 and CD44 with MDR1, MCT1 and MCT4 in androgen non-responsive, metastatic CaP cells lines as well as in high grade primary CaP tissues. Over-expression of CD147, CD44v3-10, MDR1 and MCT4 was significantly associated with CaP progression. Co-localization of CD147 and CD44 with MDR1, MCT1 and MCT4 in tumor and stromal cells further highlights the importance of cell invasion in regulating drug resistance in the progression of CaP. Our results suggest that selective targeting of CD147 and CD44 either alone or combined with chemotherapeutic agents, may be effective in reducing multidrug resistance, increasing drug sensitivity and controlling hormone refractory CaP disease.