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Gene expression profile comparison between bone metastatic and non-metastatic prostate cancer cell lines

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Prostate cancer is currently the most commonly diagnosed cancer and the second leading cancer death in man in western countries. About 90% of patients with advanced prostate cancer have metastases in bone which is currently untreatable and is the main cause of morbidity in these patients. It has been suggested only the so called metastasis initiating cells are responsible for the formation of bone metastases, via acquisition of a mesenchymal phenotype through epithelial to mesenchymal transfer (EMT). Studies have shown that P2 receptors were involved in prostate cancer cell growth. A recent study has suggested that alterations in the expression P2 of receptors were involved in EMT in MDA-MB-468 breast cancer cells. Similar gene expression alterations of P2 receptors may also present in bone metastasis initiating prostate cancer cells. In this study, we performed Taqman quantitative RT-PCR to reveal the gene expression profile of three human prostate cancer cell lines with different ability to form bone metastases, including LNCaP (non-bone metastatic to bone), C4 2B4 (derivative of LNCaP but forming osteoblastic metastasis), and PC3 (forming osteolytic metastasis). Total RNA was isolated from cultured cells using the Promega ReliaPrep RNA Cell Miniprep system. First strand cDNA was synthesized from 200ng RNA in a 20 μ l reaction system containing 250ng Promega random primer, 100mM dNTP, and 200U Invitrogen SuperScript III. Individual qRT-PCR was performed using equal amount of synthesized first strand cDNA. Results showed that RNA (CT value < 35) of P2X4, X5, X7, Y₁, Y₄, Y₁₃, and Y₁₄ receptors were expressed in all the three cell lines. Among these P2 receptors, *P2RX4* is the most highly expressed (CT value ~24), while *P2RY14* receptor is the least expressed (CT value ~32). The relative gene expressions (Δ CT) were also compared between non-bone metastatic cell line LNCaP and bone

metastatic cell lines C4 2B4 and PC3, using *GAPDH* as the endogenous control. Results showed that both C4 2B4 and PC3 expressed significantly higher level of *P2XR3* but lower level or no expression of *P2XR2*, *P2YR6*, and *P2YR11* compared to LNCaP, while the more mesenchymal-like PC3 cells express significantly higher levels of *P2YR1* (~8 fold) than both LNCaP and C4 2B4. In conclusion, this study has provided consistent and further detailed gene expression profile of prostate cancer cell line compared to previous studies. It also highlighted that alterations in gene expression of certain P2 receptor subtypes (*P2X2*, *P2X3*, *P2Y₆*, and *P2Y₁₁*) may be correlated with the ability of prostate cancer cells to metastasize to bone and a mesenchymal phenotype.