Isolation and Analysis of Bone Marrow Disseminated Tumor Cells from Patients with Localized Prostate Cancer

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Background: Bone marrow disseminated tumor cells (DTCs) are thought to be a source of many prostate cancer recurrences. With the exception of one recent report, investigators have been able to detect these cells and correlate their presence with clinical outcome for decades. However, isolation (rather than mere detection) of prostate cancer DTCs from human bone marrow has been problematic. Fluorescence activated cell sorting (FACS) has been used in other fields to detect, isolate and subsequently analyze rare cells from bone marrow, for example hematopoietic stem cells.

Methods: Therefore, we developed FACS based methods for prostate cancer DTC isolation after testing two approaches. In the "lenient" approach, we isolated viable bone marrow mononuclear cells which were CD45 negative and expressed any level of EPCAM; ($EPCAM^{dim\ or\ high}$ / $CD45^-$). This population was present at a frequency on the order of 1 cell in 100 and was also present in bone marrow from healthy donors. Therefore, we concluded that this strategy isolated primarily normal bone marrow cells. In the second "stringent" approach we isolated cells which were CD45 negative, only expressed EPCAM at a high level and were also negative for three additional markers; CD235a (erythroids), alkaline phosphatase (osteoblasts) and CD34 (HSCs), so that the population was $EPCAM^{hi}$ / $CD45^-$ / $CD235a^-$ / $AlkPhos^-$ / $CD34^-$.

Results: At a threshold of ≥ 2 cells in a million, the stringent population was present in 16/42 patients with localized prostate cancer, 1/2 patients with metastatic prostate cancer and 1/7 normal donors. As validation that these cells are tumor derived, we compared their frequency in patients with localized prostate cancer to each patient's risk of prostate cancer recurrence after radical prostatectomy as determined by the CAPRA-S score – a composite of pre-treatment prostate specific antigen (PSA) plasma concentration, Gleason score, extra-capsular extension, surgical margins, seminal vesicle invasion and lymph node invasion. The frequency of the cells selected by stringent criteria significantly correlated with the CAPRA-S score by the Spearman rank correlation test ($r = 0.66$, $p = 0.0001$). As expected, there was no correlation between CAPRA-S score and the frequency of cells selected by the lenient criteria. Furthermore, the stringent cells expressed mRNA for the prostate cancer markers, *KRT8*, *KLK3* (PSA) and *PCA3,* three orders of magnitude higher than in the lenient cells. Lastly, gene expression was analyzed in lenient vs. stringent cells by RNA-Seq which showed increased expression of prostate cancer marker genes in the stringent population from 3 of 8 localized prostate cancer patients.

Conclusions: DTCs are present in the bone marrow of a significant minority of patients with localized prostate cancer. FACS based methods are able to isolate viable cell populations containing these DTCs, which can be used for gene expression analysis.

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