Metabolic Analysis of Slowly Cycling Melanoma Cells

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Introduction. *In vivo* and *in vitro* investigations of malignant melanoma revealed substantial tumor heterogeneity [1]. Sub-populations of cells with tumor initiating property have been identified in melanoma [2] breast [3], colon [4], and brain [5] cancers. The chromatin-remodeling factor JARID1B was identified as a molecular marker for this sub-population in melanoma [6]. JARID1B-positive cells exhibit high tumorigenicity, self-renewal capacity and resistance to treatments. High expression of JARID1B suppresses cell proliferation. This property of melanoma initiating cells was utilized in our earlier work where we developed a method to image this subpopulation base of label retaining effect [7]. The objective of the current study was to delineate a difference in the metabolism between slowly cycling sub-cell populations and bulk (normal cycling) tumor cells. This information would be important for development of treatment against this sub-cell population.

Methods. In the present study we used modified human melanoma cell line MW3734 that expresses green fluorescent protein (GFP). GFP expression is driven by JARID1B expression. WM3734 cells were grown in T175 flask till 95% confluence. Approximately $5x10^7$ cells were harvested and sorted for GFP positive (slowly cycling cells) and GPF negative (cells with normal/fast cell cycle). Sorted cells were transferred back into medium for 2-3 days for recuperation. Ethanol/chloroform cell extracts were obtained using a method described previously [8]. Extracts were lyophilized and re-dissolved in 0.6 ml D_2O. The solution was then transferred to a 5-mm NMR tube, and a capillary tube containing sodium 3-(trimethylsilyl)-[2,2,3,3,-$^2H_4$]-1-propionate (TSP) was inserted as an external reference standard for quantitation. High-resolution ^{1}H NMR spectra were acquired on a 11.7 T superconducting magnet interfaced to a Varian console. The following parameters were used: 90 flip angle, TR 5 s, and 5120 averages. A pre-saturation pulse was used to suppress the water signal.

Results: Cells were sorted (Figure 1A) and cells extract were analyzed by NMR. Figure 1B shows typical spectra of the same amount of normal/fast cycling (JARID1B negative) cells (top), slowly cycling (JARID1B positive) subpopulation (middle) and unsorted WM3734 cells (bottom). High lactate and choline signals were detected in slowly cycling (middle) and unsorted (bottom) cells while normally/fast cycling cells reviled relatively small signals from these metabolites. Integral of signal intensity was calculated and compared between different cells' populations (Figure 1C). Amount of lactate in unsorted cells was taken for 100%. Lactate produced by slowly cycling subpopulation was almost 70% of total signal (unsorted cells), while normal/fast cycling cells produced only ~30% of total lactate. Similarly, much higher signal from choline was detected in unsorted and JRID1B-positive cells compared to normal/fast proliferating cell fraction.

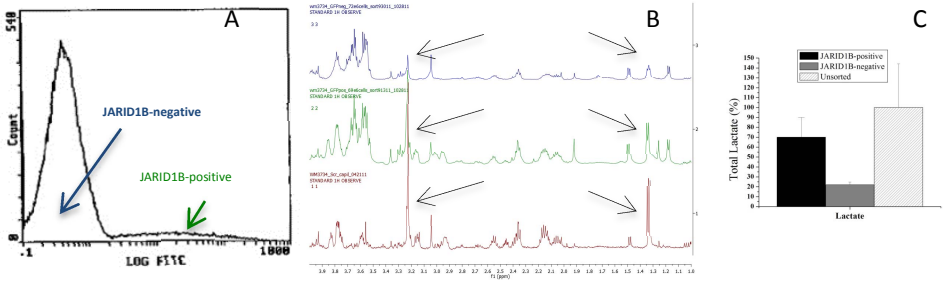

Figure 1: (A) Cell sorting diagram: peak on the left is JARIB1B-negative cells, small peak on the right is JARID1B-positive cells (B) NMR spectrum of JARID1B negative (top), JARID1B positive (middle) and unsorted cells (bottom). (C) Normalized lactate concentration in JARID1B-positive, -negative and unsorted melanoma cells.

Discussion: Low efficacy of existing methods of treatment of many human cancers has been attributed to the existence of cancer stem cells. Slowly cycling melanoma cells exhibit several stem like propertise. In the present study we determined the difference between slowly cycling sub-poppulation and bulk tumor cells. Our experiments showed that almost 70% of lactate in unsorted melanoma cells comes from slowly cycling sub-population. Taking into acount that slowly cycling cells is only 1-5% of total cell population, this result can explain the longliving parados discovered by Warbrug: detection of high lactate in tumor tissue with no evidence of abnormality in TCA cycle and oxidative phosphorilation components of cancer cells.

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