Novel triple-positive markers identified in human non-small cell lung cancer cell line with chemotherapy-resistant and putative cancer stem cell characteristics

ABSTRACT

Through the specific identification and direct targeting of cancer stem cells (CSCs), it is believed that a better treatment efficacy of cancer may be achieved. Hence, the present study aimed to identify a CSC subpopulation from adenocarcinoma cells (A549) as a model of non-small cell lung cancer (NSCLC). Initially, we sorted two subpopulations known as the triple-positive (EpCAM+/CD166+/CD44+) and triple-negative (EpCAM−/CD166−/CD44−) subpopulation using fluorescence-activated cell sorting (FACS). Sorted cells were subsequently evaluated for proliferation and chemotherapy-resistance using a viability assay and were further characterized for their clonal heterogeneity, self-renewal characteristics, cellular migration, alkaline dehydrogenase (ALDH) activity and the expression of stemness-related genes. According to our findings the triple-positive subpopulation revealed significantly higher ($P<0.01$) proliferation activity, exhibited better clonogenicity, was mostly comprised of holoclones and had markedly bigger ($P<0.001$) spheroid formation indicating a better self-renewal capacity. A relatively higher resistance to both 5-fluouracil and cisplatin with 80% expression of ALDH was observed in the triple-positive subpopulation, compared to only 67% detected in the triple-negative subpopulation indicated that high ALDH activity contributed to greater chemotherapy-resistance characteristics. Higher percentage of migrated cells was observed in the triple-positive subpopulation with 56% cellular migration being detected, compared to only 19% in the triplenegative subpopulation on day 2. This was similarly observed on day 3 in the triple-positive subpopulation with 36% higher cellular migration compared to the triple-negative subpopulation. Consistently, elevated levels of the stem cell genes such as REX1 and SSEA4 were also found in the triple-positive subpopulation indicating that the subpopulation displayed a strong characteristic of pluripotency. In conclusion, our study revealed that the triple-positive subpopulation demonstrated similar characteristics to CSCs compared to the triple-negative subpopulation. It also confirmed the feasibility of using the triple-positive (EpCAM+/CD166+/CD44+) marker as a novel candidate marker that may lead to the development of novel therapies targeting CSCs of NSCLC.

Keyword: CSCs; Triple-positive markers; EpCAM+/CD166+/CD44+ cells; A549 cell line; NSCLC