Lung cancer, the most common cancer in humans, causes more than 1 million deaths worldwide each year. A growing body of evidence suggests that lung cancers contain a subpopulation of cancer cells responsible for the initiation, propagation, and metastasis of lung cancers. These cells are termed cancer stem cells (CSCs) or tumor initiating cells. Compared to other common cancers such as those arising in the breast and colon, lung CSCs remain poorly characterized. The research outlined here aims to identify novel cell surface markers for isolating lung CSCs, which is essential for characterization of these important cells and holds the promise of leading to novel prognostic tests and therapeutic interventions for this disease.

Few lung CSC markers have been identified to date. Two recent reports suggest that CD133, together with the pan-epithelial marker EpCAM, can be used to isolate human lung CSCs. In these reports, double positive cancer cells (CD133+EpCAM+) were shown to be able to initiate lung tumors in immunodeficient mice, suggesting that this subpopulation is indeed enriched for lung CSCs. We have been prospectively acquiring primary human lung cancers and analyzed the expression of a number of surface markers using flow cytometry. CD133 expression was not detected on most of the human lung cancers we have profiled. In addition, CD133 was also absent in several lung cancer xenografts we established from these samples. These findings suggest that while CD133 may be a good CSC marker for a subset of human lung cancers, its utility will be limited. It is therefore of utmost importance to identify novel lung CSC markers that can apply to a larger fraction of lung tumors.

To identify novel candidate lung CSC markers, we analyzed published microarray data from both human lung cancers and normal lung cells, and identified six promising candidates that had interesting expression patterns and/or were CSC markers in other tumor types. After examining the expression of these six markers in a dozen primary lung cancer samples, we found that four of the markers could reproducibly divide lung cancer cells into distinct subpopulations and were expressed on all of the tumors we analyzed. Interestingly, in the few CD133 positive lung cancer samples, the cell population with highest expression of our novel markers was also highly positive for CD133, suggesting that our new markers and CD133 can identify the same CSC subpopulation in these tumors. Additionally, each of the four markers was expressed in the human lung cancer xenografts we established from our surgical specimens, suggesting that lung CSCs maintain expression of these markers in the animal model. Taken together, our preliminary data suggest that we have identified four novel CSC markers for human lung cancers.

The research proposed in this application aims to verify and validate the four novel CSC markers. We will sort primary lung cancer cells based on the expression of these markers using flow cytometry and will test their stem cell activity both in vivo and in vitro. By definition, CSCs must be able to initiate tumors in immunodeficient mice, while the
remaining cancer cell subpopulations must not. We will therefore test if our novel markers can enrich for tumor initiation in mice. In vitro, we will use a three-dimensional culture system, which allows formation of spheres from normal lung/progenitor cells, to culture the various cancer cell subpopulations identified by our markers. Putative CSCs are expected to also display sphere-forming activity in this assay. Also, we will test differentiation capacities in vitro for the various cancer cell subpopulations identified by our markers. Prospective CSCs are able to differentiate to mature cell types in vitro. This research plan will allow us to test the hypothesis that our novel markers identify lung CSCs. If successful, our markers will serve as a foundational tool for the study of lung CSCs and will greatly accelerate the characterization of these important cells. Ultimately, these results will help us to not only better understand human lung cancer, but also design new therapies targeting lung CSCs in order to improve treatment outcomes for this devastating disease.

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