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Could TK1 Suppress the Immune System and Promote Tumor Development?

JUNE 21, 2019 BY ADMIN

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Cancer is a widespread disease that affects millions of people worldwide and in many cases is difficult to treat. In order to find new and better treatment, it is important to understand how this disease progresses. Thymidine Kinase 1 (TK1) is a recognized biomarker that is upregulated in cancer cells compared to healthy tissue. Although this protein is normally found inside the cells (cytosol), it becomes upregulated on the surface of malignant cells. Moreover, recent research shows that TK1 expression is further upregulated in breast cancer tissues that undergo a transition from stationary (epithelial) cells to more mobile (mesenchymal) cells. Here we hypothesize that TK1 may play a role in tumor migration and invasion. This study utilized siRNA to knock down TK1 expression in A549 lung cancer cells and then examined the cells’ ability to migrate across a biological barrier. This migration, or invasion potential, is a unique quality of cancer cells that is the root cause of metastasis, where secondary cancer growth occurs away from the initial site. Metastasis contribute directly to the high mortality rate of cancer patients. Our results showed that TK1 expression does have a significant impact on the invasion potential of cancer cells.

We purchased A549 lung cancer cell line for this experiment. Some of the cells were grown as normal and others were subject to small interfering RNA (siRNA). SiRNA technique allows one to temporarily eliminate the production of certain proteins inside the cell. In this case, we had three different siRNAs (58, 59, 60) that targeted TK1. In order to find out whether siRNA worked we subjected the cells to flow cytometry. Through the use of antibodies, this technique allows one to confirm presence or absence of cell surface proteins. Finally, we examined how TK1 levels on cell membrane impacted cell motility and invasion potential. Cells were placed into a small chamber and were allowed to migrate to a different chamber filled with a chemoattractant (a chemical that attracts the cells and thus induces their movement). The chambers are separated by a barrier that mimic tissues in the body a cancer cell would potentially pass through during metastasis.

To assess TK1 silencing in cells subjected to siRNA we stained them with antibodies targeting this protein. Flow cytometry (Figure 1) analysis revealed that A549 cells treated with siRNA 60 showed no appreciable reduction in TK1 on the surface. Cell treated with siRNA 59 showed a 16% reduction and those treated with siRNA 60 showed the most significant reduction of 94%. Then the cells were subjected to an invasion study. It aimed to assess their motility and invasion potential in vitro. Figure 2 shows that cells treated with siRNA 59 or siRNA 60 behaved statistically equal to the cells not treated with any siRNA. However, cells treated with siRNA 58, which had a large decrease of surface TK1 (94%), showed a significant reduction in invasion potential.
Figure 1 – Quantification of flow cytometry data showing how siRNA impacts surface TK1. The blue bar shows the control (no siRNA added). The purple bar represents siRNA 60 and the green bar represents siRNA 59. No appreciable reduction in surface TK1 can be seen. However, siRNA 58 (red bar) demonstrates significant silencing of the protein on the surface (93.82% reduction).

Figure 2 – Invasion assay with A549 malignant lung tissue. Y-axis represents the invasion potential and X-axis corresponds to the time interval in hours. Blue (no siRNA), Green (siRNA 59), and Purple (siRNA 60) are statistically equal. However, Red (siRNA 58) shows a significant reduction in the invasion potential of the malignant cells.

Over the last several decades TK1 has shown promise as a diagnostic and prognostic biomarker for several types of cancers. Its membrane expression is also a unique event that occurs only in malignant tissues. Even rapidly dividing healthy cells, such as colon cells, do not show surface TK1 expression. This raises the question as to what advantage this provides the malignant cells. Studying the invasive potential of cells with and without surface TK1 has shown significant dependence on its surface expression. These findings showing TK1 surface expression as a potential factor in cell motility and invasive potential help shed light on cancer behavior and illuminate a potential target in the clinic.
Because of the differential expression in malignant cells, TK1 could potentially be used as a target in immunotherapy. Specific drugs could, also, be used to inhibit metastatic events in cancer cells. Many cancers, such as breast cancer, are fairly treatable at the initial site but are much more deadly once metastasis have developed away from the initial site. If surface TK1 targeting agents can be developed, these metastatic events could potentially be halted, leading to more effective and long-term cancer treatment techniques that have lower mortality rates and lower rates of recurrence. The battle against cancer has been, is, and will continue to be arduous and complicated. Despite this, we are optimistic that as more is discovered about this disease, more effective treatments can be developed that will improve clinical outcomes.