Genetic manipulation by inserting green and red fluorescent colors in human brain tumor cells
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Cancer is a scary diagnosis that leaves one with an unfortunate feeling since the road to health is long or worse, may never be reached. Cancer can arise, for reasons still unknown, in any part of the human body, such as the brain. Normal brain development starts off with neural stem cells, which are cells that have the ability to develop into any type of brain cell. As development continues, the neural stem cell becomes specialized (also called differentiated) cells with a certain function. These differentiated brain cells are critical for structural functions and cellular signaling and communication. Unfortunately, abnormalities in signaling pathways happen and gives cancer a dangerous green “go” light allowing it to develop into a full blown disease.

Glioblastoma multiforme (GBM) is the most malignant primary brain tumor in human beings. GBM patients are left with a poor prognosis of only 14.6 months even after advanced treatment, which includes surgery, radiation and chemotherapy. Defining a successful GBM treatment is difficult since the tumor consists of many different GBM cell types of which some are resistant to treatment. These cells, known as cancer stem cells (CSC), are thought to be the culprits causing tumor re-occurrence and relapse meaning that the tumor has the ability to come back even after its removal using cancer treatment. A new treatment option is to target the CSCs since this is the limiting factor of the current treatment strategy in order to help GBM patients survive. Thus, a critical start to the fight against GBM is to better understand the underlying mechanism of CSCs. As a result, the main focus of this project was to establish a method that enables direct observation of differentiation of glioblastoma cancer stem cells as a potential tool for efficiently screening molecular drug possibilities.

How was this done? The human DNA can be edited using a tool known as the CRISPR (clustered regularly interspaced palindromic repeats)-Cas 9 system. This tool is used to make precise cuts and insertions in the DNA. I used the CRISPR tool to insert a green fluorescent protein tag (GFP) to the stem cell marker nestin, which was found to have lower protein expression in the differentiated cancer state. Thus, whenever the nestin gene is transcribed from DNA into RNA and then translated into protein or “expressed,” there will be a green color, which indicates that the cancer stem cells are still activated. I also used the CRISPR tool to insert a red fluorescent protein tag (RFP) to the differentiation marker, glial fibrillary acid protein (GFAP). GFAP was found in this project, in agreement with other studies, to have higher protein expression in the differentiated cancer state. Whenever GFAP is expressed, there will be a red color, indicating the cancer stem cell has differentiated and is more susceptible to drug treatment. Imagine this tagging of the differentiation process as a stop light where green means GO or that the cells are resistant to treatment and red means that the CSCs are STOPPED or that all GBM cells, even the resistant ones can be eliminated. I found that the bone morphogenetic protein 4 (BMP4) forced CSCs to differentiate and as a result, can be used as a "pre-treatment" to standard GBM treatment in order to eliminate the resistant CSCs. BMP4 will be used in the future to differentiate CSCs under direct observation using live cell imaging as a potential tool for molecular screening. The hope is that as cancer cells differentiate there will be a shift in green to red color indicating that cells are being pushed into a state where they are more susceptible to treatment. In the long run, GBM patients would have a chance at a more positive prognosis; a longer life that is free of glioblastoma.