Inflammatory protein converts glioblastoma cells into most aggressive version

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A prominent protein activated by inflammation is the key instigator that converts glioblastoma multiforme cells to their most aggressive, untreatable form and promotes resistance to radiation therapy, an international team lead by researchers at The University of Texas MD Anderson Cancer Center reported online today in the journal *Cancer Cell*.

The discovery by scientists and physicians points to new ways to increase radiation effectiveness and potentially block or reverse progression of glioblastoma multiforme, the most common and lethal form of brain tumor.

"We know that the mesenchymal (MES) subgroup of glioblastoma cells is the most aggressive subgroup clinically," said co-senior author Ken Aldape, M.D., chair and professor of Pathology and Kenneth D. Muller Professor in Tumor Genetics. "This paper shows that the NF-kB pathway causes cells to change to that MES subgroup."

This conversion leads to radiation resistance.

"The pathway we identified serves as an escape mechanism for tumors," said lead author Krishna Bhat, Ph.D., assistant professor of Pathology. "In newly diagnosed patients, even before treatment, these cells already are poised to meet radiation therapy challenges."

NF-?B-driven cell change starts outside the tumor

NF-?B activation is stimulated by inflammation, which occurs in the tumor cell's microenvironment.

"The shift of tumor cells to a MES type, characterized gene expression associated with invasion and new blood vessel formation, leads to radiation resistance," said co-senior author Erik Sulman, M.D., Ph.D., assistant professor of Radiation Oncology. "This suggests blocking the inflammatory response to make tumors more sensitive to standard radiation treatment may improve outcomes for patients."

Standard care for glioblastoma is surgery, followed by radiation and chemotherapy and then treatment with temozolomide. An estimated 23,270 people will receive a glioblastoma diagnosis in 2013 and about 14,000 people will die of the disease. Median survival is about one year.

Cell line, mouse model show something missing

"No one really knows how glioblastoma progresses from its early stages because 90-95 percent of cases are diagnosed without prior history of a lower grade glioma," Bhat said. Of these about 50 percent belong to the MES subgroup. A previous study had shown that glioblastomas with a proneural (PN) type, have a much better prognosis. But these less-aggressive tumors tend to recur as the aggressive MES subtype after treatment."

Research at MD Anderson and other institutions identified the two distinct cell types based on genes expressed by each. "We haven't known what makes a cell evolve into the MES subtype," Bhat said.

Bhat took cells from 41 human glioblastoma samples and placed them in

cell cultures. Of these, 33 developed into neurospheres, cells that take on stem-cell like characteristics.

Microarray analysis of gene expression in the 17 fastest -expanding cell cultures divided them into two distinct groups: one cluster similar to the MES subtype and the other the PN subtype.

They analyzed expression of four genes commonly expressed by each subtype to see how the cultured cells matched up to their parental tumors.

Cue the surprise

All but two of the cell lines (70 percent) that originated from MES tumors lost their MES characteristics and acquired a PN signature. These results do not match the human experience, Bhat noted. Glioblastoma cells don't retreat from an aggressive to less aggressive state.

Either something in the cell culture system favored enrichment of the PN state, or most glioblastoma neurospheres exist in the less-aggressive PN state, and something in the tumor microenvironment triggers their reversible differentiation into the MES state.

Placing the PN cells cultured from MES tumors in mice did not restore those cells to the parent tumor's more aggressive type.

Different responses to radiation treatment

The researchers implanted glioblastoma sphere culture grafts from MES and PN types in mice and then treated them with radiation.

Those with the PN type had increased survival after treatment compared

to controls and had a dramatic accumulation of cells (48 to 78 percent) stuck in a specific phase of the cell cycle caused by irradiation, which lead to massive cell death.

Irradiating MES tumors produced no or minimal survival advantage and the percentages of cells arrested by treatment was reduced to 19-25 percent. The MES cells also showed an enhanced ability to repair damage caused by irradiation.

The Cancer Genome Atlas project for glioblastoma had previously found that genes in the TNF? receptor family and the NF-?B pathway are enriched in MES subclass tumors that also express high levels of the surface receptor CD44.

This team found the exact same pathway had been turned on in the MES cells in their study.

Subsequent experiments found:

- Treating PN cells with TNF? caused a dramatic increase in CD44 expression. This effect could be reversed by impeding NF-?B.
- Pretreating PN cells with TNF-alpha before radiation treatment greatly reduced cell damage.
- NF-?B controls three main transcription factors known to produce the MES cell signature and forces conversion to MES by inducing those factors.

MES cells, CD44 levels, NF-kB activation predict human radiation response

In a cohort of newly diagnosed glioblastoma patients, the team found that those in the MES subgroup, with high levels of CD44 and activated

NF-?B had poorer response to radiation and reduced survival.

A separate analysis of PN to MES transition in human tumors showed that regions with higher MES signatures had greater invasion by immune cells called macrophages /microglia – elements of the glioblastoma microenvironment – than did PN areas.

"We know we have to control inflammation in this disease," Bhat said. NF-?B is known to play an important role in promoting inflammation in multiple cell types.

"Surprisingly we found that activation of NF-?B was prevalent in the MES subtype even before surgery and radiation, which in turn can cause inflammation and further activation of NF-?B."

Bhat is investigating downstream targets of NF-?B that promote radiation resistance in glioblastoma.

Inhibitors of NF-?B are in clinical trials for inflammatory and autoimmune diseases, Aldape noted.

"One can imagine a clinical trial in which patients are evaluated for MES status and given an NF-?B inhibitor if they have the MES subtype. You can look at improving radiation response, and also whether you can reverse the MES subtype," Aldape said.

Provided by University of Texas M. D. Anderson Cancer Center