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Killing zombies: Senolytic therapy in pilocytic astrocytoma

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Intratumoral heterogeneity represents a major roadblock to curing brain cancer, as most therapies are not able to target all molecular subclones or phenotypically specialized subpopulations. Many past studies have focused on how effectively radiation, standard chemotherapy, and other agents kill stem-like or better differentiated tumor cells, and how the local microenvironment modulates therapeutic resistance. In the current issue, Selt et al. investigate a less-studied population of tumor cells—those showing a senescent phenotype—in pilocytic astrocytoma.[1] Importantly, they report that small molecules can effectively induce the death of senescent cellular elements, which can make up a large fraction of the neoplastic mass in these low-grade gliomas.

Cellular senescence, a type of irreversible growth arrest, was first described by Hayflick in cultured fibroblasts, and has subsequently been implicated in general aging and a wide range of other conditions.[2] Senescent cells are characterized by not only a lack of proliferation, but also variable combinations of activated DNA damage response, increased expression of proteins associated with cell cycle arrest, altered telomeres or chromatin, and secretion of soluble factors that modulate the local microenvironment.[2]To address problems associated with aging, "senolytic" drugs were developed based on functional and expression studies which suggested specific vulnerabilities in non-neoplastic senescent cellular populations.[3] Bcl-2 family inhibitors such as navitoclax represent one popular class of senolytic agents, and are increasingly being tested for removal of senescent cells in cancer.[2, 4]

Pilocytic astrocytoma are the commonest type of pediatric low-grade glioma, and molecular alterations activating BRAF represent the most frequent molecular drivers.[5] Proliferation is relatively low overall, and it was recognized over a decade ago that BRAF activation could promote oncogene-induced senescence in these tumors.[6] While this means many pilocytic tumors are clinically somewhat indolent, it also makes them difficult to target using standard therapies which primarily kill dividing cells. Mortality in pilocytic astrocytoma patients is relatively low, but morbidity can be quite high, and therapies which remove non-proliferative tumor cells in neurologically sensitive locations are clearly needed.

A major roadblock to the development of improved pediatric low-grade glioma therapies has been the paucity of preclinical models suitable for drug testing, at least in part due to senescence of neoplastic cells in culture.[7] The Milde group has previously shown that a surgical pilocytic astrocytoma specimen could be successfully cultured by adding an inducible construct encoding SV40 large T antigen, and that silencing its expression results in cellular senescence.[8] MAPK inhibitors were also tested in their initial study of this line, designated DKFZ-BT66. The group subsequently used DKFZ-BT66 to identify a role for the senescence-associated secretory phenotype in pilocytic astrocytoma, and performed some initial experiments using the senolytic compound navitoclax.[9] In this issue of *Neuro-Oncology*, an expanded number of lines are used, and the therapeutic focus is shifted more firmly to the potential clinical utility of senolytic agents.[1]

Selt et al. present 3 new patient-derived pilocytic astrocytoma cell lines with inducible SV40 largeT antigen, which when combined with DKFZ-BT66 result in a collection of three models with KIAA1549-BRAF fusions, and one with a BRAF p.V600E mutation. Additional characterization of all 4 lines is presented, including broad transcriptome and methylome profiling. Interestingly, the *in vitro* tumor models cluster near to the primary tumors from which they are derived.

A number of BH3-mimetic drugs were tested, and those with the strongest reported affinities for BCL-XL were most effective in targeting senescent pilocytic astrocytoma cells. Specifically, 5 small molecules with strong binding to BCL-XL had nanomolar range effects in three of the 4 lines, while 9 other standard chemotherapies and MEK inhibitors only killed these cells at much higher concentrations. Reductions in cell number and relative metabolic activity were not entirely

specific to senescent cultures, but in the 3 responsive lines changes were much more prominent in senescent states as compared to proliferative ones in which SV40 large T antigen was expressed.

Sensitive lines showed caspase-3 activation and increased apoptosis in senescent cells. The single line in which senescent cells were resistant to navitoclax and other drugs targeting BCL-XL had an altered gene expression signature which may explain its different response. Additional evidence for the importance of BCL-XL came from its induction in models grown under senescent conditions. BCL-XL levels were also found to be higher in primary pilocytic astrocytoma specimens as compared to normal cerebellum.

Other groups have also begun to investigate if targeting senescent subpopulations in different types of brain tumors by BCL-XL inhibition might help improve clinical outcomes. For example, Rahman et al. recently reported that senescent glioblastoma cells show selective vulnerability to BCL-XL inhibition.[10] However, given the large percentage of senescent cells in pilocytic astrocytoma and other BRAF-driven low-grade gliomas, the new work by Selt et al. represents a therapeutic advance of particularly great potential significance.

One critical issue will be extending the elegant but purely *in vitro* preclinical testing they present to *in vivo* models, which are also challenging to generate for low-grade gliomas. Additional questions best answered using a combination of *in vivo* model systems and the analysis of primary tumor material include how the local microenvironment might modulate senescent phenotypes, if they can alter the effectiveness of senolytic drugs, and how to most effectively combine senolytic therapies with more standard approaches to treatment.

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