Using BRAFV600E as a marker of autophagy dependence in pediatric brain tumors

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Autophagy inhibition is a potential therapeutic strategy in central nervous system (CNS) tumors. The BRAFV600E mutation is known to affect autophagy. Our studies indicate CNS tumor cells with BRAFV600E mutant cells (but not wild type) display high rates of induced autophagy, are sensitive to autophagy inhibition, and display synergy when chloroquine is combined with the RAF kinase inhibitor vemurafenib or standard chemotherapeutics. Our studies also indicate chloroquine can improve vemurafenib sensitivity in intrinsically resistant cells and in a patient with induced-vemurafenib resistance. These findings suggest CNS tumors with BRAFV600E are autophagy-dependent and that identification of BRAFV600E may be a marker to identify pediatric patients with the best potential response to autophagy inhibition.

CNS tumors are a leading cause of death in children with cancer. Development of new effective therapies is often difficult due to the relatively small number of pediatric patients compared to adults with CNS tumors. In light of this, proper patient selection for trials is key. There are more than 40 clinical trials recruiting or recently completed, evaluating the benefits of inhibiting autophagy in the treatment of adult cancer but none, as yet, in children. None of these studies are using any genetic markers to select the patients who would most benefit from autophagy inhibition.

The link between autophagy, a potential cancer cell survival mechanism, and different genetic mutations is becoming stronger. This is especially clear with the link of autophagy and the BRAFV600E mutation. Initially studied in melanoma, the importance of BRAFV600E has expanded to include a number of cancers such as lung cancer, multiple myeloma, and certain leukemias. Most recently, there has been an expansion of understanding of the importance of BRAFV600E in pediatric CNS tumors. The number of children identified as harboring this mutation has increased with improved clinical testing. Presence of the mutation in pediatric CNS tumors is also linked to the risk of increased mortality. Given the clear importance of the BRAFV600E mutation in children with CNS tumors, and the known link between the mutation and autophagy, we evaluated the role of autophagy in pediatric CNS tumors with the mutation.

Initial studies of autophagy in pediatric CNS tumors suggested no significant role for inhibition in enhancing chemosensitivity. But, when the tumor cells contain the BRAFV600E mutation, it becomes clear that this subset of tumors behaves very differently than wild-type (WT) cells in regard to their propensity to activate autophagy and the importance of autophagy for cell survival. Evaluation of BRAFV600E cells demonstrated a higher induction of autophagy under stress compared to BRAFWT cells. This suggests BRAFV600E cells rely more on autophagy for survival than WT cells. Further analysis supports this conclusion. When autophagy is inhibited in BRAFV600E CNS cells, either genetically or pharmacologically, there is a robust decrease in the viability of the cells not seen in WT cells. This suggests BRAFV600E tumor cells are especially dependent on autophagy compared to their BRAFWT counterparts.

Keywords: brain tumors, pediatric, autophagy, BRAF, chloroquine

Abbreviations: BRAF, B-Raf proto-oncogene; serine/threonine kinase; CNS, central nervous system; WT, wild type.

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autophagy using chloroquine with small molecule inhibition of \( \text{BRAF}^{V600E} \) using vemurafenib, there is a synergistic effect resulting in even greater tumor cell kill. Thus, clinically safe and available drugs for modulating autophagy and treating CNS tumors can be rapidly translated into pediatric clinical trials where the drugs display synergy in \( \text{BRAF}^{V600E} \) tumors, but not in \( \text{BRAF}^{\text{WT}} \) tumors. This synergy is not limited to vemurafenib; it was also seen with other commonly used chemotherapy agents, demonstrating the potential for effectiveness in multi-agent therapy regimens.

The clinical evaluation of BRAF inhibitors in pediatric patients has recently started, although there are a number of clinical case reports showing the potential in targeting such tumors. But, as has been seen in adult patients, there is a great risk of developing resistance to these therapies. Recently published work has shown the potential for using autophagy inhibition to reverse resistance to BRAF inhibitors in melanoma. Our study demonstrates that the synergistic potential is present in primary tumor samples that are inherently resistant to vemurafenib.

Based on this preclinical evidence, we are also able to report on the first case using deliberate autophagy inhibition to improve a patient’s response to vemurafenib. In this case, the patient’s \( \text{BRAF}^{V600E} \) ganglioglioma of the brain stem developed resistance to vemurafenib following 11 mo of therapy. The addition of chloroquine resulted in dramatically improved clinical symptoms as well as renewed tumor response radiographically. Interestingly, it was also shown that the effect is driven by synergy between the 2 drugs as the patient experiences tumor regrowth and clinical decline when she is treated with either drug as a single agent.

The importance of \( \text{BRAF}^{V600E} \) and autophagy can now, therefore, include pediatric brain tumors. The ability to show synergy between autophagy inhibition and RAF inhibitors was not limited to patients treated de novo and appears to continue in the presence of RAF resistance. This is different than has been seen when using combination RAF and MEK inhibition, where recently reported clinical studies have shown that once a patient develops resistance to RAF inhibition, there is not as great an effect with the addition of MEK inhibition.

Defining the role of autophagy in the treatment of brain tumors is particularly important in pediatrics because of the increased risk and impact of long-term, therapy-related side effects compared to adults. Our study has demonstrated the potential of using the \( \text{BRAF}^{V600E} \) mutation to identify patients with tumors that are especially dependent on autophagy for survival, and thus more sensitive to autophagy inhibition. There are widely available screening tests available using immunohistochemistry to determine which patients carry the mutation, making routine testing both rapid and feasible. Using a personalized medicine approach to target not only BRAF but also autophagy will help to design clinical trials to target those patients who will most benefit from this intervention.

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No potential conflicts of interest were disclosed.

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