Molecular interactions of the BRAF V600E mutant with the macroautophagy machinery in colorectal cancer cells

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The effect of tumorigenic mutation in the process of autophagy was studied in this project. BRAF is a serine/threonine kinase implicated in cell growth and proliferation. It is mutated in approximately 10% of all colorectal cancers, linked to poor prognosis. The V600E mutation of BRAF is the most prevalent. Autophagy, a process for degradation and recycling of cellular components, is known to be involved in tumorigenesis. Survival tests on Caco-2 cells overexpressing (HA)-tagged $BRAF^{WT}$ or mutated $BRAF^{V600E}$ suggested changes in survival after starvation. Therefore, we wanted to explore whether BRAF and its interactors were linked to macroautophagy in colorectal cancer cells.
The experiments were conducted using methods based on affinity purification coupled with mass spectrometry. These investigations led to the general observation that V600E cells displayed less enrichment of known interactors than WT cells. However, as known interactors were not systematically found in all the experiments, interpretation of the data was difficult.
Additionally, an autophagy assay was performed to determine whether BRAF overexpression affected autophagy flux. This assay indicated that BRAF overexpression might lead to higher MTOR activity without an apparent reduction of autophagy flux.

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