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Enhancing tumor selectivity of a picornavirus virotherapy

Oncolytic viruses that selectively target tumor cells are a promising cancer therapy and are thought to work not only via direct lysis and destruction of tumor cells but also through recruitment and activation of the host's anti-tumor immune response. While there are a number of naturally occurring viruses that preferentially replicate in cancer cells and have otherwise limited effects in human tissue, the real therapeutic promise lies in genetically engineered viruses. Through genetic engineering, researchers have been able to create a variety of viral phenotypes and thereby affect their selectivity for cancer cells, ability to infect and proliferate, and interaction with the innate and adaptive immune system. The authors conducted a study in which they examined the picornavirus Theiler's murine encephalomyelitis virus (TMEV), which causes a disease in mice that is similar to human poliovirus. There are two major subgroups of TMEV: One virus causes chronic encephalitis that persists in the central nervous system and includes Daniel's strain (DA), while the other, which includes the GDVII strain, has enhanced neurovirulence and causes a severe acute myelitis that is often fatal. The two subgroups share extensive homology, with most of the differences being within the virus capsid regions. The authors created a number of engineered viruses by inserting portions of the GDVII genome into the genomic backbone of the less virulent DA. They then examined these engineered viruses in in vivo murine cancer models in which human melanoma cell lines were implanted into the flanks of mice. They demonstrated that while intratumoral injection of the wild-type DA was not able to inhibit outgrowth of the tumors, the chimeric viruses that included the capsid from the more virulent GDVII virus were able to significantly inhibit tumor outgrowth and increase overall survival. Flow cytometry studies on dissociated tumor tissue demonstrated that oncolytic viral therapy caused an increase in intratumoral CD8+ T-cells, while the absolute number of CD4+ T-cells did not change. In addition, flow cytometry showed that 30 percent of the cytotoxic T-cells were specific for a virus antigen. Overall, this study highlights the technique of engineering viral vectors to create an oncolytic virotherapy and demonstrates that the recruitment and stimulation of the adaptive immune response in a proinflammatory environment plays an important role in oncolysis. The findings also show the importance of the picornavirus capsid, suggesting that enhancing viral infectivity and replication could improve therapeutic efficacy. In fact, the alterations that occur in the surfaces of cancer cells, including changes in protein expression, post-translational modification, and surface glycosylation, may play a role in determining the tumor specificity of viral particles. Therefore, rationally designing and engineering a variety of oncolytic viruses could advance cancer treatment strategies.

Bell MP, Pavelko KD. Enhancing the tumor selectivity of a picornavirus virotherapy promotes tumor regression and the accumulation of infiltrating CD8+ T-cells [published online ahead of print January 28, 2016]. *Mol Cancer Ther.* doi:10.1158/1535-7163.MCT-15-0459.

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Sequencing multiple genes in patients with early onset and familial breast cancer

A sizeable minority of breast cancers may result from heritable mutations, the best known of which are in the *BRCA1* and *BRCA2* genes. Genetic counseling and testing for *BRCA* mutations is recommended for breast cancer patients with early onset or those who have a strong family history. If identified, cancer-related mortality in these patients can be reduced with more vigilant screening and prophylactic treatment. However, other medium- to high-penetrance genes have been shown to cause familial breast cancer syndromes. Therefore, a panel of multiple genes may be necessary to more fully understand predisposing genetic factors. The authors conducted a study in Taiwan in which 133 breast cancer patients who had early onset breast cancer, bilateral breast cancer, or a strong family history of breast or ovarian cancer underwent germline sequencing with a panel of 68 genes that had known or potential association with hereditary cancer syndromes. The mean depth of sequencing was 195 reads, and more than 90 percent of coding exons had a depth of at least 50 reads. Of the 133 patients, 30 (22.6 percent) had germline heterozygous mutations known to be deleterious in the genes examined. While most of the observed germline mutations were seen in *BRCA1/2* (nine in *BRCA1,* 11 in *BRCA2*), mutations were also found in *RAD50* (two patients), *TP53* (two patients), and *ATM, BRIP1, FANCI, MSH2, MUTYH,* and *RAD51C* (one patient each). In addition, while the study identified more than 14,000 other missense mutations in the examined genes, after bioinformatics and protein structural analysis, most were classified as benign, and only 12 were classified as variants of unknown significance. The authors found that mutation prevalence was higher, but not significantly so, in younger patient groups. Family history of male breast cancer was very significantly associated with germline mutations. Germline mutations were also seen significantly more frequently in patients with triple-negative breast cancers. Overall, this study highlights the use of a large gene panel for cancer-risk assessment and is the first report of such in the Asian-Pacific region. Mutation rates in BRCA1/2 were similar to those previously reported in Western countries, and 7.5 percent of patients had germline mutations in other cancer-related genes. However, questions still remain regarding the most effective panel design. While next-generation sequencing technology allows for sequencing many cancer-related genes in parallel, it is not known how many or which ones should be the highest priority for familial breast cancer. In addition, for many genes, it is not known which germline variants are of clinical significance and exactly how they affect cancer risk. Therefore, one must consider how to report known deleterious mutations in genes for which there is little consensus on clinical follow-up or how therapeutic approaches should be modified. Finally, one should be careful when reporting variants of unknown significance due to the potential for excessive patient anxiety. The authors concluded that performing routine germline gene testing in a subset of patients may be more effective than BRCA1/2 testing alone and that this study sheds light on possible gene panels that could be used in the future.

Lin P-H, Kuo W-H, Huang A-C, et al. Multiple gene sequencing for risk assessment in patients with early-onset or familial breast cancer [published online January 27, 2016]. *Oncotarget.* doi:10.18632/oncotarget.7027.

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