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Oncolytic Reovirus: Susceptibility and Resistance in Cancer Cells

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In the past decade several promising candidate viruses have been tested for potential utility in the treatment of human cancer. Although detailed mechanisms for the cytolysis of cancer cells vary in each case, a common theme is that the viruses naturally or after modification demonstrate the potential to replicate preferentially in and kill transformed cells compared with healthy cells and tissues. An example is reovirus, which is a common and relatively benign double stranded RNA virus that can be isolated from the respiratory and gastrointestinal tract of humans. Remarkably, these viruses also display oncolytic potential with a wide array of tumor types, and especially against those cancers bearing activated Ras oncogenes, which leads to the enhancement of protein synthetic pathways upon which viral replication depends. Here we provide evidence for the utility of reovirus in the treatment of fibrosarcoma and other tumors, conditions under which resistance to reovirus may arise, and strategies to overcome this resistance.

Historically, reovirus has been recognized for many years as displaying striking cytocidal activity when it infects certain types of transformed cells (Duncan and Stanish, 1978; Hashiro *et al.*, 1977). The underlying basis for reoviral oncolytic activity remained unknown until it was shown that transformed cells containing oncogenic Ras signaling pathways were preferentially susceptible to reovirus infection (type 3 Dearing strain) *in vitro* and *in vivo* (Coffey *et al.*, 1998; Kim *et al.*, 2007; Norman and Lee, 2005; Strong *et al.*, 1998). As Ras gene mutations are frequently observed in various types of human cancers (Duursma and Agami, 2003), these findings have led to the current use of reovirus in phase I and II clinical trials (Norman and Lee, 2005; Stoeckel and Hay, 2006) in Canada, the UK and the US.

Given its very real clinical potential, it is therefore important to understand fully the diverse factors that govern the susceptibility and resistance of cancer cells *vs.* normal cells to reoviral infection. We first asked whether transformed cells carrying mutationally activated Ras genes could acquire resistance to reovirus. For this study (Kim *et al*., 2007) we used human fibrosarcoma HT1080 cells that contain

an activated N-*ras* gene. Normally these are killed efficiently with reovirus, but we found that variant cell lines emerged after prolonged exposure to reovirus that were no longer killed. The resistant cell lines still expressed the active N-*ras* gene and showed elevated GTPase activity, but displayed reduced levels of cathepsin enzyme, a lysosomal protease required by reovirus for its entry to the cytoplasm where it replicates. Importantly, we found that the reovirus resistant cells could still be killed by exposure to other chemotherapeutic agents or to oncolytic adenovirus, indicating that apoptotic pathways remained fully functional. These results demonstrate that additional pathways, beyond Ras activation, are necessary for efficient reoviral oncolysis, and furthermore that combinational or sequential use of viral and/or chemotherapeutic agents may show enhanced effectiveness in cancer therapy.

Interestingly, the virus-resistant fibrosarcoma cells derived in these experiments were persistently infected with a modified variant strain of reovirus that had accumulated several mutations compared with the wildtype strain. These mutations included a premature stop codon in the gene encoding the sigma-1 coat protein of the virus, which normally is thought to be essential to the viral infection process. However, we found that the mutation merely caused a mild attenuation of the virus, such that it retained the ability to efficiently kill fibrosarcoma cells *in vitro* and *in vivo*, but showed reduced damage to healthy tissues and undifferentiated stem cells, especially in immunodeficient animals in which wildtype reovirus can cause myocarditis, neural damage and other disorders. We also found that the damage caused by reovirus could be completely blocked by a cathepsin inhibitor, both *in vitro* and *in vivo*. Many cancer patients show varying degrees of immunosuppression as a result of disease progression or chemo- or radiotherapy, and our results may be helpful in developing strategies for efficient viral therapy in such challenging circumstances.

Thus our studies are contributing to a better understanding of the underlying basis and range of activity for viral therapy in cancer, should furthermore lead to the more accurate identification of patients who may benefit from this approach, and finally may point to promising avenues for future strategies to optimize viral or combination therapies.

References

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