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“Characterization of antioncogenic effects of autonomous parvoviruses in permanent and tissue preserving short time cell culture of solid tumors”

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Autonomous parvoviruses are small nuclear replicating DNA viruses, non- or weakly pathogenic in adult animals, and do not appear to integrate in host chromosomes during either lytic or persistent infections. Some of them were found to preferentially replicate in and kill *in vitro*-transformed cells and reduce the incidence of spontaneous and implanted tumors in animals. Because of their natural oncotropic and oncolytic properties, parvoviruses are considered as potential antitumor vectors.

With the ultimate goal to assess whether human tumors can successfully be treated with wild type parvoviruses or derived recombinant vectors, the ability of the oncolytic autonomous parvovirus H-1 to replicate in and kill human colon tumor cells was investigated. Since the tendency in cancer therapy is to apply approaches based on specific modifications that occurred in tumor genes, the analysis of the antineoplastic potency of H-1 parvovirus was extended to colorectal cancer samples obtained by surgery immediately after operation. The tumor samples were grown in three-dimensional structures, a technique known as histoculture, allowing the application and evaluation of therapies, specific for individual cancers.

Five established colon cancer cell lines were tested in parallel and characterized for their susceptibility to H-1 wt virus infection and killing – HT29, SW480, COLO320DM, LOVO and SW948. All analyses performed with the colon cancer cells were compared with the effect of infection on NB324K cells, the standard virus-producer and the most sensitive to virus-induced killing.

The analyzed colon cancer cell lines were found to be highly heterogeneous in their responses to virus infection. They were observed to differ in their sensitivity to H-1

parvovirus toxicity, suggesting that the repertoires of altered genes in these cell lines are diverse. Two of these cell lines - COLO320DM and HT29, were qualified as highly permissive to H-1 infection and a strong correlation between virus induced culture growth inhibition, cytotoxicity, parvoviral DNA amplification and production of progeny virions was observed. A low competence for virus DNA replication was detected in SW480 cells, as the effects of growth inhibition and killing were weak, despite their proficiency for viral gene and protein expression, and their capacity to produce progeny viruses. Finally, the colon cancer cell lines LOVO and SW948 were deficient for H-1 virus processing and subsequently highly resistant to H-1 killing. The latter two cells lines were found to have low capacities to take up the virus, an observation that explains their poor permissiveness to virus infection. In similar studies with cells from other tumors (liver, mammary or glioma), internalization/ receptor deficient cells were never identified.

Considering the sensitivity of the cell lines to H-1 parvovirus infection, the analyses showed a tendency for increased tumor susceptibility with increased tumor progression. In particular, SV40 large T-antigen over-transformed SW480 colon cancer cells infected with H-1 parvovirus were more susceptible to killing than the parental cells. E-cadherin cell adhesion protein expression in the over-transformed cells was reduced compared to the precursor cell, indicating tumor progression and lower differentiation. A similar pattern of E-cadherin expression was observed in COLO320DM, characterized as very permissive to H-1 killing. On the contrary, LOVO and SW948 cell lines showing the strongest surface expression of E-cadherin were highly refractory to H-1 virus induced killing.

Colon tumor samples obtained immediately after surgical intervention were also subjected to H-1 virus infection in attempts to evaluate their *in vivo*-like response to H-1 parvovirus oncolytic activities, as these samples were cultured in their original three-dimensional native structure. Unfortunately, metabolic activities and cell viability measured in the infected tissue samples responded only weakly to virus infection compared with the strong reduction of the mentioned parameters when the tissue was treated with 5-FU, a drug conventionally used to treat colon tumors. Yet these observations confirmed the oncoselectivity of the virus, since a multiplicity of cell types are presented in the original tumors (normal and cancer cells).

The tumor status of the tissue samples in view of genetic alterations must also be considered, since the observations with the cell lines pointed to an increased susceptibility to parvovirus killing with increased cell transformation. Furthermore, considering the characteristic genetic instabilities established for these colon cancer cell lines, it was concluded that colon cancers with CIN (chromosomal instability) or MSS (microsatellite stability) are more permissive to H-1 killing than MSI (microsatellite instable) colon cancers,

although more tumors have to be analyzed to confirm this statement. Nevertheless, more genes playing a role in H-1 virus-induced tumor cell killing also need to be identified.

The identification of different genetic phenotypes among colon tumors with observed significant levels of permissiveness to H-1 killing will help to employ the right approach when a decision on the gene therapy treatment has to be made. As HNPCC colon tumors frequently have MSI and the tested cell line with MSI (microsatellite instability) was found strongly resistant to H-1 infectivity, this subclass of colorectal tumor is not supposed to be an appropriate target for H-1 gene therapy. On the contrary, CIN (chromosomal instability) cell lines and the observed permissiveness to H-1 infection suggest that FAP (familial adenomatous poliposis) and sporadic colon cancers with a high frequency of CIN can be potentially targeted by H-1 parvovirus gene therapy with expected positive therapeutic effects.