UniversityHospital Heidelberg

GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

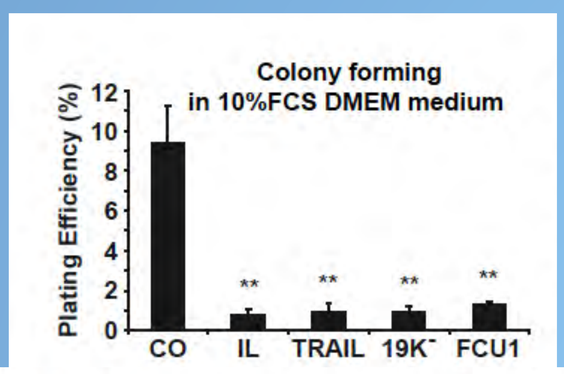
Targeting of pancreatic cancer stem-like cells by MSC-delivered oncolytic adenoviruses

Adam Kaczorowski[1,2], Katharina Hammer[3], Li Liu[1,2], Jury Gladkich[1,2], Dirk Nettelbeck[3], Ingrid Herr[1,2]

1 Molecular OncoSurgery, University of Heidelberg and German Cancer Research Center, Heidelberg, Germany
2 Department of General Surgery, University of Heidelberg, Heidelberg, Germany
3 Helmholtz-University Group Oncolytic Adenoviruses, German Cancer Research Center and Heidelberg University Hospital, Department of Dermatology, Heidelberg, Germany

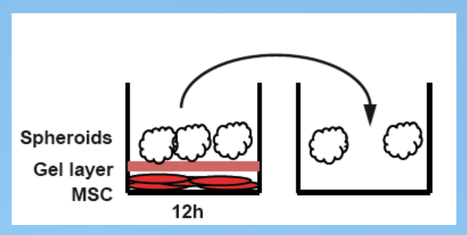
Introduction: Pancreatic cancer is highly resistant to conventional therapies. It is thought that this is due to cancer stem cells (CSC), a small subpopulation of tumour cells, which promote resistance and invasiveness. Oncolytic adenoviruses represent a novel agent for the elimination of CSC. They are designed to infect and replicate selectively in cancer cells. Upon cell lysis viruses are released and can infect new cells. For an efficient delivery to the tumour mesenchymal stem cells (MSC) are used as virus carriers, being infected in vitro and injected into the patient. MSC show a homing ability towards tumour tissue and integrate into the stroma where they should infect and ultimately destroy the tumour cells.
Aims*:* We studied the elimination of CSC by oncolytic adenoviruses and the invasion ability of infected MSC *in vitro* and *in vivo*.
Methods: MIA-PaCA2, an established pancreatic cancer cell line with a high amount of CSC were used. MSC were isolated from human bone marrow by Ficol centrifugation and used in low passages. Cells were infected with adenovirus in 2%FCS DMEM medium and incubated for 2 hours. Different virus constructs were used. All used constructs contain a gen for GFP, some viruses are additionally armed with genes for drugs like TRAIL (induces apoptosis in malignant cells) or FCU1. A virus which is deficient in an anti-apoptotic protein and should be more aggressive was also used (19K-).

Oncolytic adenoviruses inhibit colony-formation



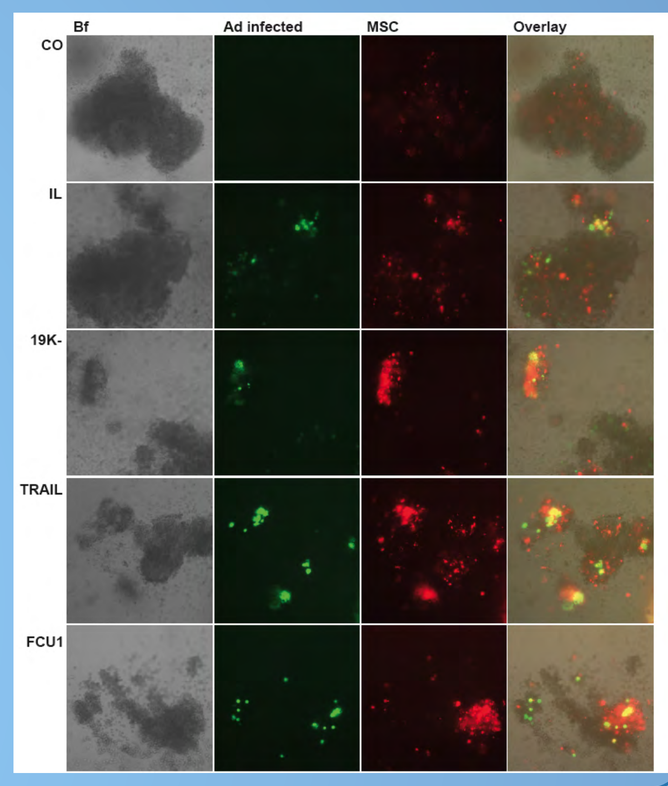
MIA-PaCa2 cells were seeded 24h post infection at low density. Growth of colonies from single cells is an marker for CSC and correlate with the tumor initiating potential of a cell line.
Colony formation is strongly reduced in infected cells, which shows infection and elimination of CSC by the virus. No significant difference could be observed between the different virus types.

Oncolytic adenoviruses do not prevent invasion of MSC to tumour spheres

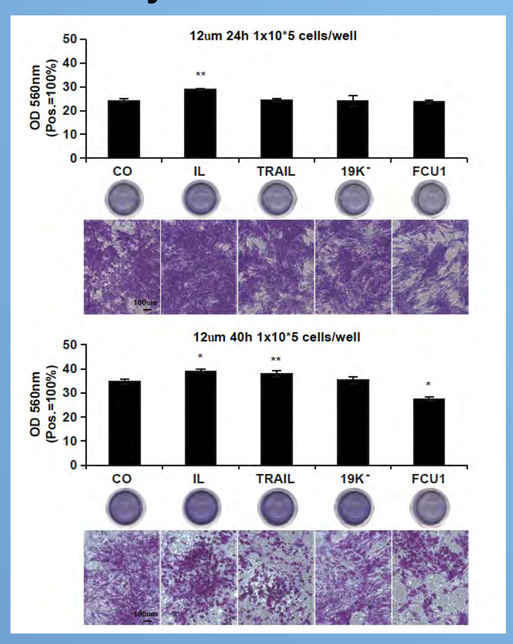


For using MSC as virus carriers the infection should not interfere with their homing ability. To demonstrate this attached MSC were marked with a red fluorescent dye, infected with different viruses and coated with a Matrigel/ collagen layer. MIA-PaCa2 spheroids were put on top of the gel layer and incubated over night. Afterwards the spheroids were put into a fresh well and the presence of invaded red fluorescent MSC was evaluated.

Infected MSC (which express GFP) could be observed in every group. This shows invasion of tumour spheroids by adenovirus infected MSC. The rate of invading cells was highly variable. Furthermore the presence of cells fluorescing only in red shows a strong migration of uninfected cells. Therefore we will qualify the migration potential of infected/uninfected MSC. For this a single cell suspension will be created out of spheroids after invasion and the cells will be centrifuged on slides via cytospin. Ratios of uninfected/infected MSC present on the slides can then be estimated.

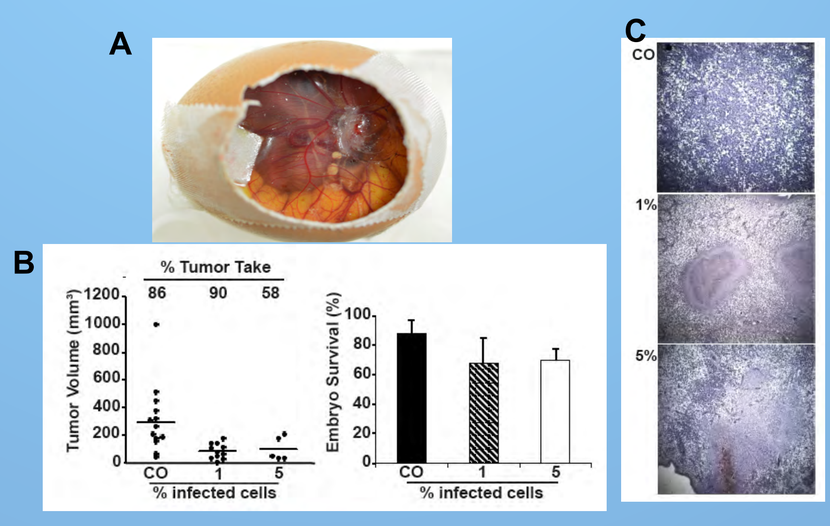


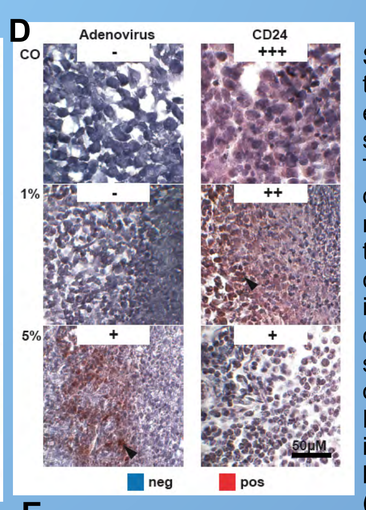
Migration of MSC is not inhibited by oncolytic adenoviruses

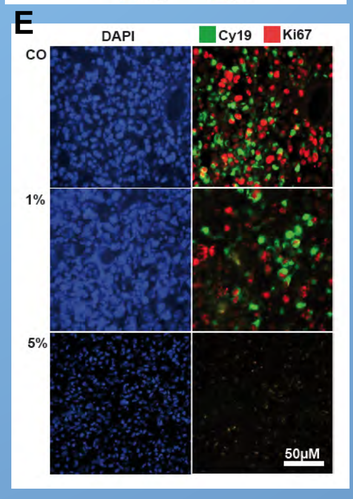


MSC infected with different oncolytic adenoviruses migrated through the pthe bottom side of the membrane were counted and the number of migratory cells was quantified.
The MSC showed good migration after 48h. MSC from the infected groups migrated as well as uninfected olycarbonate membrane of a transwell chamber along a FCS gradient. After migration the membrane was stained and cells on control MSC, or even better, as in the case of IL and TRAIL. In contrast cells from the FCU1 virus infected group showed slightly less migration.

Oncolytic adenoviruses block tumour growth *in vivo*







For evaluation of the influence of virus infection on tumour growth and CSC characteristics in a model with a stronger resemblance to the clinical situation we used the CAM Model in fertilized chicken eggs. MIA-PaCa2 cells (containing 0/1%/5% infected cells) were grafted through a cut-out piece of the shell on the chorio-allantoic membrane inside of a silicone ring (**A**). The tumour is innervated by blood vessels from the CAM membrane and forms a stroma with chicken fibroblasts and shows similar morphology to patient tumours.
Tumours were removed after 10 days. Infected tumours showed a strong reduction in size, indicating inhibition of tumour growth by the oncolytic adenovirus (**B**). While this effect was present even at 1% of infected cells, an effect on tumour take (fraction of eggs developing tumours) appeared only with a higher number of infected cells.

Slides of paraffin embedded tumours were used for further evaluation. Infected tumours showed altered morphology (**C**). They contained clusters of dense, small cells which were never seen in uninfected tumours. Presence of the virus could be only confirmed by immunohistology in some of this clusters (**D**). Infected tumours showed also a lower presence of CD24, a marker for CSC.
Proliferation was decreased in infected tumours, as indicated by the proliferation marker Ki67 (**E**).
Infection of tumours with an oncolytic adenovirus resulted in severe changes in morphology, even at only 1% infected cells. This and the reduction of CSC markers and proliferation *in vivo* demonstrate that oncolytic adenoviruses have a great potential as therapeutic agents for elimination of pancreatic CSC.

Results: We could demonstrate the effect of oncolytic adenovirus infection on CSC *in vitro*. Infection lowered the tumour initiating potential considerably. We also examined the migration and invasion potential of MSC after infection. We could prove the invasion of tumour entities by infected MSC and their migration. Migration did not appear to be negatively influenced by infection. But an high number of uninfected MSC present in invaded tumour spheroids raises the possibility of most migratory cells being uninfected. Therefore a confirmation of the ratio of infected cells in migrating/invading populations has to be done. Infection of tumours *in vivo* considerably reduced growth, proliferation and CSC characteristics. This indicates an efficient repression of pancreatic tumour growth by our therapy.
Outlook: The infection and elimination of CSC is done on primary pancreatic cancer cell lines, with promising first results. Additionally we are evaluating the rate between infected/uninfected MSC during migration. Migration of infected MSC will also be studied in vivo. For this marked MSC will be grafted on the CAM of tumour-bearing eggs and presence of MSC in the tumour tissue will be evaluated.