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Novel therapeutic approaches based on lactaptin action

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Motivation and Aim: Immunotherapeutic approaches become a new hope for cancer treatment. Clinical success was demonstrated for adoptive cell transfer of T cells expressing chimeric antigen receptors (CARs) as well as for oncolytic viruses. However, both technologies are needed to be improved. Lactaptin was discovered as a molecule specifically inducing death of various cancer cells *in vitro* and *in vivo*. So we propose to use lactaptin transgene for armoring CAR NK-cells and vaccinia virus.
Methods and Algorithms: We have engineered double recombinant vaccinia virus (VV) coding human granulocyte-macrophage colony-stimulating factor (GM-CSF) and apoptosis-inducing protein lactaptin (VV-GMCSF-Lact). To engineere "armored" CAR NK-cells secreting an anticancer peptide lactaptin at the first stage we designed lentiviral constructs allowing stable transduction of human cell lines with cassettes encoding two secreted forms of lactaptin.
Results: VV-GMCSF-Lact activated a set of critical apoptosis markers in infected cells: phosphatidylserine externalisation, caspase -3, -7 activation, DNA fragmentation, up-regulation of pro-apoptotic protein BAX and efficiently decreased mitochondrial membrane potential of infected cancer cell. Investigating immunogenic cell death (ICD) markers in cancer cell infected with VV-GMCSF-Lact we demonstrated VV was efficient in calreticulin and HSP70 protein externalisation, cellular high-mobility group box-1 (HMGB1) decreasing and ATP secretion. The analysis of antitumor activity against advanced MDA-MB-231 tumor in mice revealed that VV-GMCSF-Lact delay tumor growth up to 94 %.
Lactaptin was successfully produced in HEK293T and YT cell lines. Its in vitro activity in the conditioned media was measured against a panel of sensitive cancer cells: MDA-MB-231 breast adenocarcinoma, PC3 prostate cancer and T98G glioblastoma. We evaluated that lactaptin from conditioned media showed greater than 50-fold increase in cytotoxicity compared to the recombinant lactaptin produced in *E. coli.*
Conclusion: We demonstrated that lactaptin has a great potential for improving immunotherapeutic approaches against cancer.
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