Targeted nanoparticles sustained delivery of paclitaxel biology essay

Science, Biology

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Targeted nanoparticles sustained delivery of paclitaxel to irradiated tumors

Introduction:

There are various conventional methods by which tumor cells can be killed but the problem with using these in the body is their effect on normal cells. Targeting the drugs to the particular tumor location helps in reducing their side effects. Specific cancer types have been targeted by biologics such as trastuzumab and imatinib but these are limited to cancer expressing mutations. Thus, ionizing radiation XRT that cause localized stress to cancer cells can be very useful here. In addition, targeted therapy can help in reducing side effects and helps in improving the treatment efficiency for chemotherapy. In this research article, Ralph J. Passarella et al have identified a peptide Gly-Ile-Arg-Leu-Arg-Gly (GIRLRG) and radiation-induced receptor pair that can be used for a targeted and controlled drug delivery system using nanoparticles. The authors are trying to find whether peptide

gets induced on exposure to radiation and recognize radiation treated tumors. Hence, authors are trying to investigate whether this combination can be therapeutically benefits or not.

Materials and methods:

Nude mice and GL261 murine glioma and MDA-MB-231 are the human breast cancer cell lines used for the study. Heterotropic tumor models were developed by inoculating the nude mice. Human umbilical vein endothelial cells (HUVEC) were cocultured with GL261 murine glioma cells. The cells were treated with 3 Gy XRT and incubated in streptavidin-peptide-AlexaFluor594 complex. Imaging was done by using a fluorescent microscope. Near IR images of the mice treated with 3Gy XRT and injected with peptides, were done using the IVIS imaging system with the help of labeled complexes. GL261 tumor samples treated with 3 Gy XRT / sham XRT from the hind limbs of nude mice were extracted 48 hours later treatment and Mem-PER Eukaryotic Membrane Protein Extraction Kit was used to extract the protein. The protein was incubated in the Dialysis Cassettes overnight and then with NeutrAvidin-coated agarose beads bound to biotinylated GIRLRG. The beads were washed with PBS, boiled and run on Invitrogen NuPAGE 10% gel, stained with the help of Invitrogen Stain. Bands obtained from the gel were viewed with Vanderbilt Proteomics Core through the liquid chromatography/tandem mass spectrometry technique. The extracted protein was surface modified with actin and GRP78 antibodies on a polyvinylidene difluoride membrane. KKCGGGGIRLRG peptide was added to Dimethyl sulfoxide DMSO and added to nanoparticles of

poly(valerolactoneepoxyvalerolactone-allylvalerolactone-oxepanedione) which contains epoxide and cross-linked with 1 equivalent of 2, 2-(ethylenedioxy)bis(ethylamine) per epoxide and heated for 72 hours at 340C before dialyzing. Tumors were removed from nude mice after 1 and 3 weeks of treatment (3 Gy XRT and systemic paclitaxel/paclitaxel nanoparticles with RILGGR and GIRLRG) and incubated with monoclonal antibody to paclitaxel (1: 500). Paclitaxel antibody staining and TUNEL staining were done to evaluate the apoptotic effects of the paclitaxel on tumors. Statistical analysis was also performed to correlate binding of peptide and its effect on the tumors.

Results:

In vivo biopanning method was used to screen T7 phage-based random peptide library GIRLRG and identified to be the predominant peptide recovered from irradiated GL261 mice glioma. In vitro studies using HUVECs revealed that the GIRLRG targeting peptide binds to tumor vasculature only when tumor cells were irradiated and are able to interact with HUVECs. This suggests that peptide specifically binds only tumor cells and not normal cells which avoids side effects. Also, near IR studies of the in vivo tumor model showed that the peptide preferentially binds to the radiation-treated tumors. Agarose bands coated with GIRLRG were incubated with protein extracts from GL261 tumors and removed 48 hours after irradiation. A 78-KDa band was isolated and analyzed using mass spectrometry, which confirmed it to be GRP78. The expression of GRP78 on the surface of tumors was high in case of irradiated tumors. This suggests that GRP78 is expressed as a result

of exposure to radiations. The reason for the GRP78 expression is the stress caused due to radiation to tumors. Interaction between GIRLRG and GRP78 was studied by blocking the antibodies to GRP78 in different in vitro and in vivo experiments. When the antibodies blocked GRP78, peptide GIRLRG was not able to bind GRP78 which was proved in vitro experiments and this suggests that the peptide might interact with GRP78 and help in targeted binding to tumors. Peptides were conjugated with the synthesized nanoparticles by the reaction of the cysteine present near NH2 terminus of KKCGGGGIRLRG with allyl groups present in the nanoparticles. Paclitaxel was then incorporated and biocompatibility studies were done and proven that there is no cytotoxicity related to the peptide targeted nanoparticles. The staining assays suggests that peptide targeted nanoparticles have helped in sustained and targeted release of paclitaxel which has led to apoptosis of tumor cells. The sustained release can be achieved by varying the cross linking density of the nanoparticles. However, the kinetics of the drug release is not clear. In vivo studies with nude mice implanted with breast carcinoma MDA-MB-231 and GL261 glioma, showed that the tumor tripling time was delayed by 55 days and 12 days, respectively with the use of peptide-conjugated nanoparticles under XRT, which is better as compared to non-targeted nanoparticles, unirradiated tumors and XRT treatment alone. This suggests that along with death of tumor cells, tumor growth has also been controlled. Hence, novel peptide and receptor has been identified for targeted delivery of drug using nanoparticles which can be used as a therapy for cancer.

Discussion:

Positive aspects: The authors have validated their claim of synthesizing nanoparticles with targeting peptides that recognize XRT irradiated tumor cells and thus can undergo controlled pharmacokinetics. They have followed a systematic approach, providing proof of action of these particles in delaying the tripling time of tumor cells, using in vitro and in vivo studies. Further clinical trials have to be carried out to study the effectiveness of this approach on cancer patients at various stages of cancer. Negative aspects: The problem in this drug delivery system is that the tumor cells in the organism may not be irradiated uniformly leaving some cells untreated in the tumor. Also, these paclitaxel loaded targeting nanoparticles only slows the growth of tumor cells, but not destroy them completely. Not much study has been done with the characterization of nanoparticles. Suggestions: Paclitaxel is a mitotic inhibitor and only arrests the growth of cancer cells2. Other anti-cancer drugs like methotrexate, bleomycin, etc. could be used to load into the peptide-conjugated nanoparticles instead and studied for their effects of cytotoxicity on cancer cells, instead of growth inhibition, which would be more effective for cancer treatment. Furthermore, studies could be carried out to manipulate the controlled release of drugs from these nanoparticles at the target site by controlling the intensity of radiation applied.