Radioimmunotoxin Therapy of Experimental Colon and Ovarian Cancer
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Most recent report of results to date:

To pursue the development of radiolabeled immunotoxins (RIT) for colon cancer, it was first necessary to identify an immunotoxin (IT) that could selectively kill colon cancer cell lines. Recently, our collaborators in the Vallera laboratory have observed that potent recombinant IT can be synthesized using recombinant single chain antibodies (sFv) spliced to truncated diphtheria toxin (DT) consisting of the first 390 amino acids of native DT. DT was chosen as a toxin because it is a catalytic bacterial toxin that is easily manipulated in genetic engineering studies. Also, the Vallera lab has developed new procedures for preparing the sFv fusion toxins from bacterial inclusion bodies such as DT and another good genetic engineering toxin pseudomonas exotoxin (PE) based on detergent refolding. This allows for enhanced yields and higher purity that is essential for generating the protein that will be needed for preparation of larger amounts of RIT for therapy. Many potential sFvs were considered for targeting colon cancer. The best results have been obtained with an sFv recognizing EpCam. EpCam, also known as ESA or EGP40, is a 40 kDa epithelial transmembrane glycoprotein found on the basolateral surface of simple, pseudostratified, and transitional epithelia. It has been found overexpressed on 81% of adenocarcinomas of the colon (Went *et al.* Human pathology 35:122, 2004). EpCam sliced to DT (DTEpCam) was highly potent in studies in which we measured its ability to inhibit the proliferation of the HT-29 and COLO 205 colon cancer cell lines since we measured its IC_{50} at 1-2 x 10-2 nM. Potency is important, but is also critical that DTEpCam is selective in its cytotoxicity against EpCam-expressing target colon cancer cells. The activity of DTEpCam was highly selective since irrelevant control IT that did not recognize any markers on cancer cells, did not show any activity against the same colon cancer cell lines. Also, blocking studies were performed in which DTEpCam was mixed with the EpCam sFv that was synthesized without any toxin attached. The proliferation studies showed that EpCam sFv was able to block the killing of the EpCam expressing cells by DTEpCam. An irrelevant control protein, 1D10Fc was unable to block. Together, these studies indicated that EpCam was exquisitely selective. In order to produce an IT of even greater potency, we used a toxin containing the Golgi retention sequence KDEL. The same EpCam sFv was spliced to truncated PE containing the terminal KDEL sequence. The addition of KDEL enhanced the potency of the EpCam sFv IT at least 6 logs or 1000-fold with an IC50 of 2 to 7 x 10-8 nM. This conjugate was also shown to be highly selective. Taken together, all of these studies indicate that in vitro experiments have shown that we have a highly potent IT that selectively kills colon cancer cells.

The next step was to show that the EpCam IT had the ability to inhibit the growth of flank tumors in vivo in nude mice. The same human colon tumor cells, HT29 used in the in vitro studies were injected into the flank of nude mice. Tumor cells were injected into groups of mice and when tumors reached the size of 0.5 cm3, we injected our best-performing EpCam IT called EpCamKDEL intratumorally. There was a significant drop in tumor size indicating that this agent was very effective against human colon cancer. Since the EpCamKDEL was injected intratumorally, it did not have to travel through the systemic circulation to find its target. Our next step will be to inject EpCamKDEL intravenously into

mice with flank tumors to determine if EpCamKDEL has the ability to migrate to the tumor systemically.

The next step was to radiolabel EpCamKDEL to see whether it could serve as an RIT. We radiolabeled EpCam with 111In as a surrogate for 90Y and then incubated it with HT29. The labeling efficiency was over 90% indicating that a high percentage of the protein molecules could be readily radiolabeled. However, the immunoreactivity was only 20% indicating that only 20% of those molecules were able to specifically bind antigen once they were radiolabeled. We are currently determining whether this labeling procedure is too harsh on the recombinant protein or whether some other labeling procedure might result in a higher level of immunoreactivity.

Most recent products delivered:

Vallera DA, Todhunter D, Kuroki DW, Shu Y, Sicheneder A, Panoskaltsis-Mortari A, Vallera VD, Chen H: Molecular modification of a recombinant, bivalent anti-human CD3 immunotoxin (Bic3) results in reduced in vivo toxicity in mice. Leuk Res. 29:331-41, 2005.

Todhunter DA, Hall WA, Rustamzadeh E, Shu Y, Doumbia SO, Vallera DA: A bispecific immunotoxin (DTAT13) targeting human IL-13 receptor (IL-13R) and urokinase-type plasminogen activator receptor (uPAR) in a mouse xenograft model. Protein Eng Des Sel 17:157-64. 2004.

Most recent notes concerning the project:

None

Other Project Information Sources:

Project URL:

None

Related URL at institution:

None