**BMC
Proceedings**

POSTER PRESENTATION

Open Access

Hemangiosarcoma and its cancer stem cell sub-population are effectively killed by a toxin targeted through epidermal growth factor and urokinase receptors

Jill T Schappa[1,2*], Aric M Frantz[1,2], Brandi H Gorden[1,2], Erin B Dickerson[1,2], Daniel A Vallera[2,3], Jaime F Modiano[1,2]

From São Paulo Advanced School of Comparative Oncology
Águas de São Pedro, Brazil. 30 September-6 October 2012

Background

Targeted toxins have the potential to overcome intrinsic or acquired resistance of cancer cells to conventional cytotoxic agents. We hypothesized that EGFuPA-toxin, a bispecific ligand-targeted toxin consisting of a deimmunized *Pseudomonas* exotoxin conjugated to epidermal growth factor (EGF) and urokinase (uPA), would efficiently target and kill cells derived from canine hemangiosarcoma (HSA), a highly chemotherapy resistant tumor, as well as cultured hemangiospheres, used as a surrogate for cancer stem cells (CSC).

Materials and methods

We evaluated EGFuPA-toxin activity in four HSA cell lines (Emma, Frog, DD-1, and SB), using a feline mammary carcinoma cell line (K12) and a human T-cell leukemia line (Jurkat) as controls. Hemangiospheres were grown under serum-free low adherence conditions to enrich cancer stem cells. Cytotoxicity was determined using the CellTiter96 AQueous viability assay. Specificity for cells expressing cognate receptors was confirmed using neutralizing antibodies and competitive binding assays. Relative receptor expression in target cell lines was verified using flow cytometry.

Results

EGFuPA-toxin showed cytotoxicity in each of the HSA cell lines tested at concentrations ≤100 nM that was dependent on specific ligand-receptor interactions. Monospecific targeted toxins also killed HSA cells; in this case, a "threshold" level of EGFR expression

appeared to be required to make cells sensitive to monospecific EGF-toxin, but not to monospecific uPA-toxin. The IC50 of CSCs was higher by approximately two orders of magnitude compared to non-CSCs, but these cells were still sensitive to EGFuPA-toxin at nanomolar (*i.e.*, pharmacologically relevant) concentrations.

Conclusions

Our results support the use of these toxins to treat chemoresistant tumors such as sarcomas, including those that conform to the cancer stem cell model. Our results also support the use of companion animals with cancer for further translational development of these cytotoxic molecules.
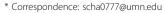
Financial support

Author details
[1]Veterinary Clinical Sciences, University of Minnesota, Minneapolis, MN 55455, USA.
[2]Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, USA.
[3]Therapeutic Radiology, University of Minnesota, Minneapolis, MN 55455, USA.

Published: 4 April 2013

* Correspondence: scha0777@umn.edu
[1]Veterinary Clinical Sciences, University of Minnesota, Minneapolis, MN 55455, USA
Full list of author information is available at the end of the article

BioMed Central