Nano-sized Polymeric Carriers for Cancer Diagnostics and Therapy

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Drugs administered to treat cancers typically distribute randomly throughout the body, resulting in a lower concentration of the drug at the tumor and some severe side effects due to the lack of specificity of the anticancer agent. Targeted drug delivery systems have the potential to offer more effective treatment with significantly reduced adverse effects through specific interactions with the intended cells. Over the last few years we have designed and synthesized various nano-sized polymeric carriers that can target both the tumor and its microenvironment (1-6). These nanocarriers can passively accumulate in solid tumors following intravenous administration by slow leakage across highly permeable neovasculature, owing to the enhanced permeability and retention (EPR) effect. Our water-soluble polymeric carriers, including polymer-drug conjugates and self-assembeled polymeric micelles, can be further equipped with targeting ligands (short peptide and oligosaccharide) specific for endothelial and cancer cells markers, to improve the efficacy of anticancer drugs and oligonucleotides and diminish non-specific toxicity in the treatment of cancer.

Active targeting of our polymer-drug (Doxorubicin, DOX) conjugates to E-selectin, overexpressed by angiogenic endothelial cells, was found very effective in improving the cytotoxicity of the targeted copolymer (P-Esbp-DOX) towards human immortalized vascular endothelial cells (IVECs) that over-express E-selectin, as compared to treatment with the non-targeted P-DOX conjugate and DOX alone (Fig.1). This study is currently being pursued to reveal the *in vivo* toxicity in tumor-bearing mice.

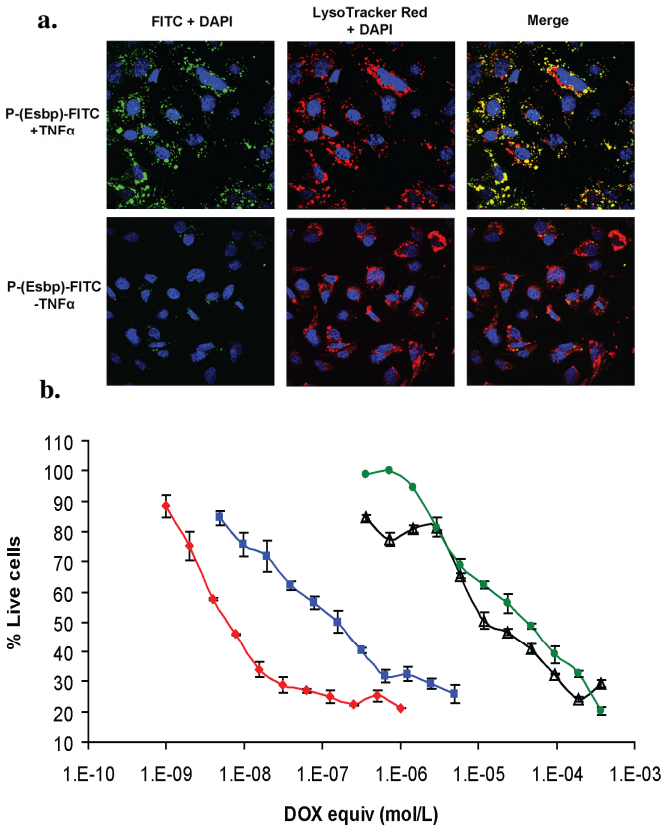

Fig.1: E-selectin targeted copolymer (P-(Esbp)-FITC) can actively target E-selectin expressing (TNFα activated) IVECs (a) and improve the cytotoxicity of the targeted polymer-doxorubicin conjugate (P-Esbp-DOX) towards E-selecting expressing IVECs, relative to non-targeted copolymers P-(Scrm)-DOX and P-DOX (b). (◆, red) free DOX drug; (■, blue) P-(Esbp)-DOX. (Δ, black) P-(Scrm)-DOX conjugate; (■, green) P-DOX

We further developed novel polymeric scaffolds to increase the drug concentration in selected sites by the use of caged cell penetrating peptide (cCPP) sequences that could trigger penetration into specific type of cells only once illuminated by light, and thus can overcome the major drawback for the CPP clinical development, i.e. the lack of cell specificity (7). We demonstrated that the neutralization of the cationic resudues of the CPP with photo-cleavable caging molecules can lead to conditional light-dependent cell penetration functionality (Fig. 2a). The cell viability profiles revealed a "light switch" in cytotoxicity of polymer conjugate bearing a proapoptotic drug $_{D\text{-}}(KLAKLAK)_2$ and cCPP (P-(cCPP)-KLAK) (Fig. 2b).

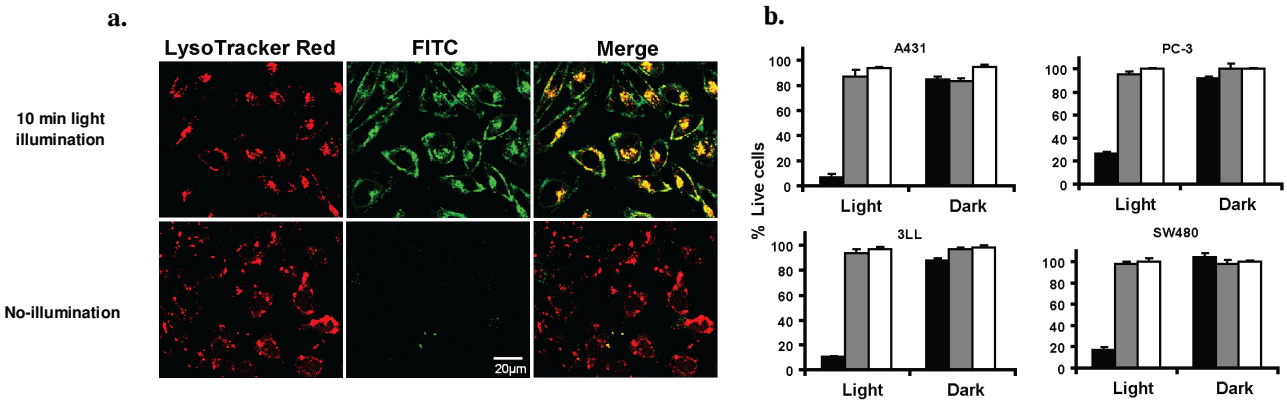

Fig.2: a). Light activated penetration of P-(cCPP)-FITC. b). Cytotoxicity due to light-induced delivery of P-(cCPP)-KLAK into different cancer cells.

We have recently generated a series of new water-soluble copolymers as diagnostic probes that could be used *in vivo* to target various diseases. These polymeric probes carry cell targeting ligans as well as multiple copies of Near Infrared Fluorescent (NIRF) dye on the same polymeric backbone. Using this approach, non-invasive tumor detection can be greatly improved (Fig.3)

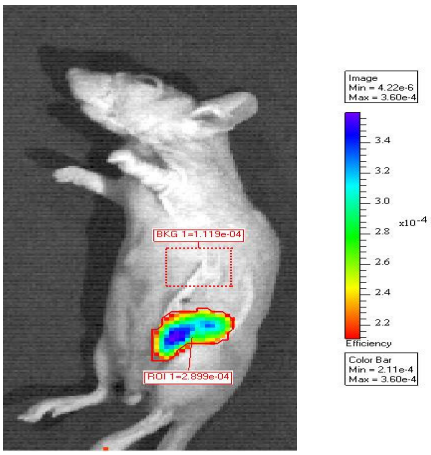

Fig. 3: In NIRF images aquired 4h after intravenous injection of P-(NIRF) probe in intra-colonic inoculated SW-480 tumors

Selected Publications:

1. David A., Kopeckova P., Rubinstein A. and Kopecek J., Design of Multivalent Glycoside Ligand for Selective Targeting of HPMA Copolymer-Doxorubicin Conjugates to Human Colon Cancer Cells, *Eur. J. Cancer,* 40: 148-57, 2004
2. Shamay, Y., Paulin, D., Ashkenasy, G., David, A. Multivalent Display of Quinic Acid Based Ligands for Targeting E-Selectin Expressing Cells. *J Med Chem*, 52, 5906-15, 2009.
3. Shamay, Y., Paulin, D., Ashkenasy, G., David, A. E-selectin Binding Peptide-Polymer-Drug Conjugates and Their selective Cytotoxicity Against Vascular Endothelial Cells. *Biomaterials*, 30, 6460-8, 2009.
4. Adar, L., Shamay, Y., Journo, G., David, A. Pro-apoptotic Peptide-Polymer Conjugates to Induce Mitochondrial-Dependent Cell Death. *Polymers for Advanced Technologies,* 22, 199-208, 2011.
5. Kopansky, E. Shamay, Y., David, A. Peptide-directed HPMA copolymer-DOX conjugates as targeted therapeutics for colorectal cancer. *J Drug Target* 19, 933-943, 2011.
6. Journo-Gershfeld, G. Israeli-Kapp, D. Shamay, Y. Kopecek, J., David, A. "Hyaluronan Oligomers-HPMA Copolymer Conjugates for Targeting Paclitaxel to CD44-overexpressing Ovarian Carcinoma. *Pharm. Res.* 29(4): 1121-33, 2012.
7. Shamay, Y., Adar, L., Ashkenasy, G., David, A. Light Induced Drug Delivery into Cancer Cells, *Biomaterials*, 32, 1377-86, 2011.