Angiotensin II-functionalized Quantum Dot Interactions with Cells

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Statement of Purpose: The angiotensin II receptor type 1 (AT_1) is one of the best investigated G Protein-coupled receptors to date. This is mostly due to its paramount role in the vascular homoeostasis as well as its high importance in the development of cardiovascular diseases like hypertension and coronary ischemia (1). In addition, the AT_1 receptor is not only overexpressed in many diseased tissues under certain pathophysiological conditions, like in the ischemic tissue after an acute myocardial infarction (2), but it is also known to play a significant role in the angiogenesis and growth of tumors (3). Therefore, it represents a promising target for drug delivery with nanoparticles. Herein, we want to investigate the feasibility of an AT_1 receptor targeting with quantum dots (Qdots), highly fluorescent probes that are coupled to angiotensin II, the physiological agonist of the AT_1 receptor.

Methods: Human angiotensin II (Ang II) was modified by introducing a sulfhydryl moiety at the N-terminus with 2-iminothiolane. Commercially available amine-terminated quantum dots 655 (Life Technologies) were activated with the hetero-bifunctional linker sulfo-SMCC to yield a thiol-reactive nanoparticle. N-terminal thiolated angiotensin II was reacted with activated Qdots to form a stable thioether bond. Unreacted maleimide groups on the nanoparticle surface were quenched with 2-mercaptoethanol. The resulting nanoparticles were purified by size exclusion chromatography and ultrafiltration. Quantitative real time PCR using LightCycler 480 SYBR Green (Roche Diagnostics) was applied to measure the cellular expression of AT_1 receptor mRNA in two cell lines, namely NCI-H295R and HeLa. To quantify the cellular association of non-modified and angiotensin II-coupled nanoparticles to NCI-H295R and HeLa cells, flow cytometry experiments were conducted. Cells were incubated with both nanoparticle species for 1 hour, subsequently washed and trypsinized. Qdots were excited at 488 nm and the cellular fluorescence emission was detected in a 661/16 nm bandpass filter. The intracellular localization of the Qdots was investigated by confocal microscopy. Colocalisation experiments were accomplished by coincubation with Alexa488-labeled transferrin. To examine the biological activity of the agonist-coupled Qdots we carried out intracellular calcium measurements with a fluorescence microscope. NCI-H295R cells were loaded with Fura-2AM (Life Technologies) for 1 followed by perfusion with respective Qdot species.

Results: Quantitative real time PCR measurements were conducted to characterize the AT_1 receptor expression on the adrenal gland carcinoma cell line NCI-H295R as well as on HeLa cells. NCI-H295R cells had a 3 orders of magnitude higher expression of the AT_1 receptor compared to the HeLa cells. Hence, the NCI-H295R cells were assumed as receptor positive cells and HeLa cells as a receptor negative control. The cellular association of the Qdots was quantified by flow cytometry. Although unmodified Qdots showed a significantly higher binding to receptor positive NCI-H295R cells compared to HeLa cells, the binding of the angiotensin II-modified Qdots was substantially higher. The peptide-modified Qdots showed no association to the receptor negative HeLa cell line (Figure 1).

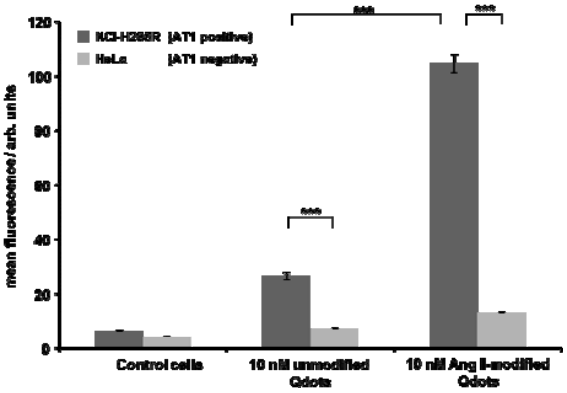

Figure 1. Nanoparticle Binding to on- and off-target cell lines measured by flow cytometry

Confocal microscopy revealed the localization of the angiotensin II-modified nanoparticles in intracellular vesicles, whereas non-modified particles showed no cellular association. In the presence of losartan carboxylic acid, an AT_1 receptor antagonist, the uptake of modified particles was greatly reduced. Coincubation studies with Alexa488-labeled transferrin revealed partial colocalization of fluorescent transferrin with modified Qdots, which further supported the receptor-mediated nature of the uptake due to the clathrin-mediated endocytosis process of both the Qdots and transferrin. Furthermore, angiotensin II covalently bound to the nanoparticle surface was still capable of inducing the phosphatidylinositol signal pathway demonstrated with intracellular calcium measurements using the fluorescent calcium chelator Fura-2.

Conclusions: We were able to show that Ang II-modified Qdots bind the AT_1 receptor and are taken up into the cells due to the receptor internalization. These findings confirm the possibility of future drug-delivery with the help of Ang II-coupled nanoparticles. Furthermore, these Qdots can be used as fluorescent probes for the tracking of the intracellular fate of angiotensin receptors.

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