Click Therapy: Novel Concept in Pretargeting Drug Delivery

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1. Basics of Click Therapy

Click therapy is a pretargeted therapeutic strategy that utilizes bioorthogonal click chemistry for treating cancers that overexpress cell surface receptors. In this strategy, pretargeting component and the drug delivery component undergo click reaction on the cell surface. Click chemistry is a concept of rapid and selective reactions between two functional groups under mild, convenient and reliable conditions. This synthetic approach is now widely used in the drug development because of high yielding, wide in scope and producing only byproducts that can be removed without chromatography. Click reactions which can be performed in biological systems without interfering regular biochemical and physiological in the living systems are called bioorthogonal click chemistry. Examples of bioorthogonal click chemistry reactions and their reaction rates are shown in the Fig. 1. However, only few click reactions can be used as bioorthogonal click reactions in living systems, since they should be biocompatible, fast and feasible in physiological conditions.

In click therapy, target-specific mAbs are delivered first to pretarget the cancer cells and are followed by the functionalized drug-carrier nanoplatform (Fig. 2). Two components react chemoselectively on the target cell surface via azide (Az) and dibenzylcyclooctyne (DBCO) (Click-1) or trans-cyclooctene (TCO) and tetrazine (Tt) (Click-2) bioorthogonal click reactions. Due to the close proximity, multiple click reactions between two components are possible on cells with high receptor density on the cell surface. These reactions result in the formation of nanoclusters, which generate tension in the cell membrane and lead to rapid internalization via receptor-mediated endocytosis.

2. Application of Click Therapy in HER2(+) Breast Cancer

Breast cancer remains one of the most devastating diseases in the world, with more than 1.5 million new cases detected every year. Approximately 20–30% of breast cancers are HER2(+) due to a gene amplification that results in high aggressiveness and a generally poor prognosis. The HER2 receptor regulates multiple physiological pathways, including cell proliferation and differentiation. The humanized anti-HER2 monoclonal antibody (mAb), trastuzumab (Herceptin®, Tz), is used as a first-line treatment for HER2(+) breast cancers. The cytotoxic mechanism of Tz includes the inhibition of the P13K/Akt and Ras/MAPK signaling pathways, leading to cell cycle arrest. However, approximately 50% of patients with HER2(+) disease do not benefit from Tz, or the disease becomes refractory to the agent, even though the HER2 level remains high.

As a proof-of-concept, click therapy has been used for the treatment of HER2(+) breast cancer. The strategy of click therapy was first evaluated by in vitro optical imaging with confocal fluorescence microscopy. Trastuzumab was functionalized with pegylated azide (Peg4-Az) groups. No significant decrease in the bind-
ing affinity of modified trastuzumab was detected in initial cell experiments. Modified trastuzumab was labeled by rhodamine and resulted in Tz(Pe4-Az)20 (Rhod)2, which was used as a prelabeling component. The degrees of conjugation and labeling were determined based on the change in molecular weights measured by MALDI-TOF and manufacturer’s protocols, respectively. They are denoted by subscript numbers in the formula. Rhodamine-labeled trastuzumab without azide functionalization, Tz(Rhod)2, was used for control experiments. As the delivery component, albumin was first prepared without paclitaxel (Px) drug conjugation. Albumin was functionalized with DBCO and labeled with Alexa Fluor-488. The resultant Alb(DBCO)15(AF-488)2 was used for initial in vitro imaging experiments. First, HER2(+) BT-474 cells were treated with the first component, Tz(Pe4-Az)20(Rhod)2, followed by the second component, Alb(DBCO)15(AF-488)2. Fixed cells were counterstained by Hoechst 33342, and wet-mounted for confocal microscopic imaging, using red and green channels. The colocalization of two components on the cell surface was observed by merging the images. The experiment at 37°C showed the formation and internalization of nanoclusters. This imaging experiment was repeated using HER2(−) MDA-MB-231 cells and no surface labeling and colocalization of components was observed. Neither colocalization nor cellular internalization by BT-474 cells was detected when unmodified trastuzumab was used as the first component. The experiment at 4°C exhibited the colocalization of two components, but no internalization. This experiment proved that (a) only cells with an overexpression of cell surface receptors provide moieties for multiple click reactions, and (b) bioorthogonal
click chemistry is the key mechanism for colocalization and in situ assembling of the components. The results also suggested that the system could be used for targeted drug delivery, and is expected to provide efficacious therapy.

For the in vitro therapeutic experiment, albumin was conjugated with paclitaxel as the model chemotherapeutic. Paclitaxel is widely used for treating advanced breast cancer and metastasis. However, paclitaxel is a highly hydrophobic compound and typically is administered as micelles made in Cremophor EL (Taxol®). Cremophor EL is relatively toxic and can induce severe hypersensitive reactions, including dyspnea and tachycardia\textsuperscript{11,12}. Alternatively, Abraxane® is a nanoparticle-albumin bound-paclitaxel drug (nab-paclitaxel) where paclitaxel molecules are physically entrapped in Alb clusters. But, Abraxane® clusters can dissociate and release chemotherapeutics en route to targets. Hence, in this approach, albumin and paclitaxel were conjugated with cleavable chemical linkers. Unlike physical entrapment of drug molecules, chemical conjugation prevents the dissociation and release of drugs before they reach the target, while the drug is released by enzymatic cleavage of linkers once the conjugate is internalized by target cells.

In the preparation of the drug-loaded albumin nanocarrier, paclitaxel was first activated by derivatization into sulfo-NHS-paclitaxel and then conjugated with albumin. The resultant drug-loaded albumin was functionalized with DBCO bioorthogonal reactive groups and labeled with Alexa Fluor® 488. The resultant Alb(Px)_2(DBCO)_2(AF-488)_2 was used as the drug delivery component. The in vitro therapeutic effect of the delivery system was studied on HER2(+) BT-474 and HER2(–) MDA-MB-231 cell lines, using Tz(Peg-Tt)_10(AF-488)_2 (Treated) or Alb(AF-488)_2 (Control), were administered i.v. 6 h after the pretargeting step and tumors were imaged after one hour post-injection. Disappearance of the cell-surface labeling and formation of internalized intracellular clusters was observed in mice treated with Tz(TCO)_6(Rhod)_2 and Alb(Peg-Tt)_10(AF-488)_2 whereas no internalization was observed in mice treated with non-functionalized Alb(AF-488)_2 as a control.

Pilot therapeutic studies were performed in two groups with three animals per group. Tz(TCO)_6(CF-680)_2 pretargeting component and Alb(Px)_2(Peg-Tt)_10(CF-750)_2 drug delivery therapeutic components were used. The injected dose for the 1st and 2nd components was 10 mg/kg and 150 mg/kg, respectively, every...
other week for seven weeks. Animals in the controlled mock-treatment group received non-functionalized trastuzumab followed by Alb(Px)2.2(Peg4-Tt)10(CF-750)2, and the untreated group was injected with saline. A significant difference between the treated, mock treated, and untreated groups was detected in the pilot studies using tumor volume as the index of response. In fact, in several animals, a complete disappearance of the tumor was observed after four weeks of treatment. The average time until the tumor volume increased by a factor of three was significantly different between the treated, control, and untreated groups.

3. Conclusion
The click therapy is a new concept of pretargeted therapy in cancers which overexpress specific receptors on cell surface utilizing the bioorthogonal click chemistry. The strategy has been used to demonstrate the HER2(+) BT-474 breast cancer models for enhanced cellular internalization of drugs, as well as cytotoxic effects of the system in HER2(+) BT-474 breast cancer cells and corresponding tumor mouse models. Two highly specific steps included in the strategy (recognition of the biological target by the antibody pretargeting component and chemoselective click reactions between the pretargeting component and the drug-loaded nanocarriers) provided highly efficient target-specific drug delivery. The formation, internalization, and cytoplasmic accumulation of cross-linked nanoclusters occur preferentially on the surface of cells where HER2 receptors are overexpressed. Accordingly, this method produces significantly less cytotoxicity in HER2(−) cells. This could potentially reduce systemic toxicity of therapy. The components of the delivery system can be further independently optimized to improve the efficacy and image-guidance capabilities. In these studies, the pretargeting component was optimized for circulation time, binding affinity, and functionality, whereas the cytotoxic therapeutic component was optimized to minimize nonspecific toxicity compared to the free drug and/or ADC formulations by adjusting the composition, linkers, and circulation time of the drug delivery nanocarrier in vivo. Promising findings obtained with click therapy provide the basis for further testing of the system using agents such as radionuclides, toxins, and interfering RNA in breast cancers, as well as in other cancer types.

References