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Personal Reflection

BRITTON CHANCE AND THE THERANOSTIC BEACON DEVELOPMENT AT PENN

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In the summer of 2000, while I was vacationing in China and getting ready to start my career in industry, I got a call from Jerry Glickson to check if I would be interested in joining the Penn Molecular Imaging Group. Jerry was very persuasive and he drew an interesting outline of the work, including the opportunity to work with one of the greatest scientific minds in the world. How could I say no to work with Britton Chance, aka "BC"? So I visited Penn in October. I remember after a long and exhausting interview day, Jerry walked me to BC's house for dinner. There he was, under the dim light, sitting at the top of the table with a bunch of high-school kids. He embraced me with open arms, held my hand and sat me down. He said that my work on developing chlorophyll-based photodynamic therapy agents was nice and that I should use these chlorophylls for cancer imaging as well since they are natural fluorophores. As the night went on, we talked about science, philosophy and life, and I was nervous and energized at the same time, just trying to catch a glimpse of his vision of the wonderful world of science. I still vividly remember his advice on staying true to the spirit of collegiality; to be always humble and willing to share and learn from others. He also asked me to keep exploring, to enjoy the fun of science and be persistent, all life-long lessons that I took deeply into my heart and continuously relied upon. By the time I got back to hotel, I realized I had hardly eaten anything except perhaps for a half bowl of Shoko's soup, but who really needs anything more after just being introduced to and taught by a giant?

When I joined Penn, BC had just started an NCI Unconventional Innovation Program featuring the synergistic development of his NIR optical imaging system and three optical imaging probe platforms [Ponzy Lu and Alan Gewirtz's mRNA beacons, Ralph Weissleder's protease probes and Jerry's NIR dye-reconstituted low-density lipoprotein (LDL)]. I picked up the LDL project with US$45,000 from BC to set up a chemistry lab in Ponzy's space that BC arranged. LDL is a nature's nanoparticle and has been used for the delivery of a number of lipophilic chemotherapeutic and imaging agents to cancer cells that overexpress LDL receptors. It is one of the smallest spherical lipid nanostructures (∼22 nm) because its apoB-100 component effectively forms an amphiphatic alpha-helical network that stabilizes the high lipid curvature. It also features extremely high loading capacity in nature (∼1500 cholesterol esters) and has a receptor recycling mechanism that is great for imaging signal amplification. So together, we developed methods for attaching a variety of

near-infrared (NIR) and MRI probes to LDL and were also able to reconstitute a number of lipophilic NIR probes into the lipid core of LDL by the Krieger procedure. Reconstitution of LDL with these dyes does not interfere with receptor binding, are free of nonspecifically exchange between the lipoprotein and the cell surface and are capable of accommodating higher payloads. This has led to the development of LDL core-loaded fluorescent photosensitizers for both photodynamic therapy and NIR optical imaging (a "theranostic" approach).[1] BC's new optical imager and his ageless redox scanner played a critical role in this effort. Later, we developed a method to reroute these lipoproteins from their natural receptors, which are expressed by numerous types of normal cells, to other receptors that are much more specific for cancer cells.[2] This broadens the utilities of these natural nanoparticles for delivery of drugs and diagnostic agents.

Another focus of my research at Penn was to develop the killer beacons for which Jerry and BC envisioned and was initially supported by Jerry's SARIP and Pre-ICMIC grants. At the time, the feasibility of using hairpin DNA molecular beacon for imaging of tumor-specific mRNAs in cancer cells was already demonstrated elegantly by Ponzy and Alan.[3] So the idea is to replace the fluorophore in the molecular beacon with a photosensitizer and to use a quencher that silences the ability of photosensitizer to generate singlet oxygen. The photosensitizer is activated by its physical separation from the quencher to generate PDT-induced cell killing, which is accomplished through hybridization with complementary mRNA of antisense-DNA that is incorporated in the beacon or by cleavage of a peptide or other linkers between the PDT agent and the quencher. To build such photodynamic molecular beacons (also called "killer beacons"), we used both pyropheophorbide (my favorite chlorophyll derivative) and bacteriochlorophyll (Jerry's favorite because of Avigdor's success in developing palladium bacteriochlorophyll PDT agents). On the other hand, the use of carotenoid as the quencher was suggested by BC because of his deep knowledge on cytochrome C, chlorophyll, photosynthesis machinery and carotenoid's photoprotective role in such system. Developing these killer beacons has proven to be a major challenge. Eventually, together with BC and Jerry and critical contribution from Brian Wilson (Toronto), we were able to demonstrate the principle of killer beacon using the protease-triggered singlet oxygen quenching and activation.[4] I think BC will be happy to know that these theranostic beacons have now progressed into mainstream PDT research by many research groups in the world.[5]

Of all my efforts on theranostic beacon development at Penn, I have to say BC's favorite beacon is the pyro-glucose, a 2-deoxyglucose conjugate of pyropheophorbide, for which I synthesized at the urge of BC and Jerry; and for which BC kept pushing me on even toward the later stages of his life. At the time, [18]F-fluoro-2-deoxyglucose (FDG) has already become the gold standard in PET imaging based on one of the biochemical "hallmarks" of malignancies, the high rate of glycolysis. In 1996, Yoshioka and coworkers in Japan developed a similar conjugate of 2DG (2NBDG) containing a smaller visible chromophore and demonstrated its internalization via GLUT1 and phosphorylation by hexokinase. BC and Henry Wagner of Hopkins tested 2NBDG's imaging potential in a brain tumor model using his redox scanner. He was convinced that a NIR version of 2NBDG could become an optical version of FDG and offer low cost and portability compared to PET imaging. Our findings suggested that the NIR fluorophores are not phosphorylated but are still retained by tumor cells expressing GLUT1.[6] More importantly, the persistent push from BC with a heavy dose of redox scanner has led to the observation of two other important findings. First, the theranostic nature of Pyro-glucose allowed us to demonstrate for the first time that redox ratio of mitochondria is an indicator for PDT response.[7] Second, by simultaneously performing redox ratio and fluorescence imaging, we demonstrated that Pyro-2DG could serve as an extrinsic NIR fluorescent metabolic index (extent of NADH reduction) for the tumors.[8] I think it is only fitting that the last study BC and I did together is Lin Li's paper on high-resolution simultaneous mapping of mitochondrial redox state and glucose uptake, which was accepted early this year.

As true to BC's impact on many people, BC has had tremendous influence not only on my career but also on my personal life. At the time that I debated with my career move, BC was the grandfather that I never had, understanding and continuously supporting my decisions. Before BC passed away, I was

eagerly planning to visit him to show him our newly discovered porphysomes,[9] truely theranostic, NIR, nanophotonics made from chlorophyll, which I knew would made BC happy. Perhaps this is BC's plan all along, leaving chlorophyll in my mind with that half bowl of soup on that magic night at his house.

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