

## ***Ex vivo* tracing of pancreatic neuroendocrine tumors with bio-conjugated fluorescent quantum dots: a paradigm of nanoparticle-based diagnostics**

**Emmanouil Giorgakis<sup>a,b</sup>, Bala Ramesh<sup>a</sup>,  
Marilena Loizidou<sup>a</sup>**

University College London; King's College Hospital NHS Foundation Trust, London, UK

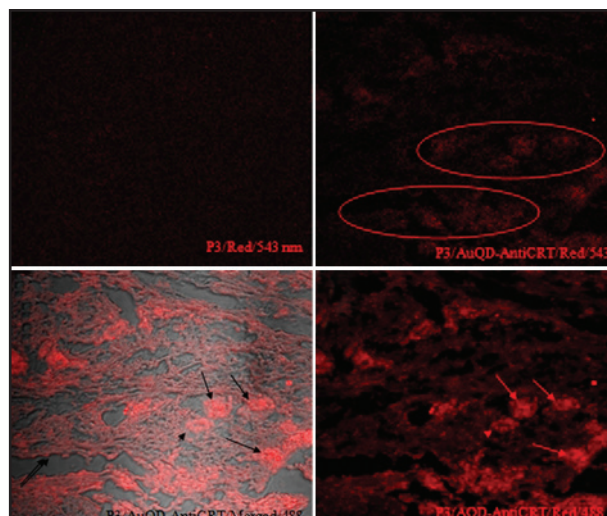
We read with great interest the article published recently by Kartalis *et al* [1]. Our study group investigated the application of fluorescence with the use of nanoparticles as a novel hybrid imaging method of pancreatic neuroendocrine tumors (pNETs). We demonstrated that a novel biomarker, namely calreticulin (CRT), is present in pNETs and may be targeted with fluorescent gold quantum dots (AuQDs).

CRT is a ubiquitous Ca<sup>2+</sup>-binding protein with chaperone activity and a rather complex relation to various cancers: its overexpression is positively associated with various solid tumors, usually as an adverse prognostic indicator [2]; however, contrary to its positive correlation to tumorigenesis and poor cancer prognosis, CRT also seems to promote immunogenic cancer cell death.

QDs are semiconductor nanocrystals with unique optical properties, such as highly tuneable fluorescence and high photochemical stability; their relatively large surface-to-mass ratio enables them to perform as antibody or drug-carriers, attributes that provide QDs with an almost unlimited potential in cancer theranostics [3]. Our group have synthesized AuQDs with functionalized groups and bio-conjugated to anti-calreticulin polyclonal antibodies (AuQD-antiCRT). These AuQDs were manufactured to emit at 800 nm on excitation in the near infrared (NIR). It can be shown that conjugated AuQDs can be targeted specifically to *in vitro* pancreatic adenocarcinoma cell lines and *ex vivo* human pancreatic lesions. With Human Research Authority approval (REC Reference: 04/q0504/1) and appropriate patient consenting, paired tissue samples from resected pancreatic specimens post-pancreatectomy were obtained and stored in liquid nitrogen. Sections were then cut on a cryostat-microtome at approximately -25°C and mounted on Vectabond coated slides for observation under laser scanning confocal fluorescence microscopy (LSCM, Nikon Eclipse TE 300). Once the fixed tissues were characterized under the LSCM, selected paired slides were incubated with AuQD-antiCRT and observed under the LSCM. The resected pancreatic specimens were further processed and evaluated by a senior histopathologist.

Among the subjects was a 61-year-old female who underwent a Whipple's procedure for what proved to be a completely resected, well differentiated neuroendocrine neoplasm, TNM stage pT3N1pMx. The photomicrographs of the pathological tissues under laser excitation before and after incubation to AuQD-antiCRT are depicted in Fig. 1. Illustrations (A) and (B) were captured by LSCM after 543 nm green laser beam excitation via a 650 nanometres/Long Pass (nm/LP) fluorescence barrier, while (C) and (D) after 488 nm blue and 650 nm/LP filtering. There was minimal signal emission after 543 nm excitation due to lower energy confirming NIR emission (A). Post-incubation with AuQD-antiCRT (B), there was NIR emission (pseudocolor) in 650/LP filter cut-off and therefore CRT expression. Images (C) and (D) also demonstrate NIR fluorescence after AuQD-antiCRT incubation, more intense upon He-Ne (488 nm). These two latter photographs also demonstrate some of the architectural appearances typical of pNETs, such as the cell nests (*single arrows*), trabeculi (*double arrows*) and gyriform patterns (*arrowheads*).

To the authors' best knowledge, this is the first pictorial demonstration of CRT surface expression on pancreatic neuroendocrine malignancy with the use of fluorescent quantum nanotechnology and tracing of pNETs with bio-stable, non-degradable fluorophores, such as the AuQDs. These findings may facilitate further *in vivo* investigation on CRT expression on pNETs and on the application of QD-based theranostics on the detection and treatment of these conditions [4]. Finally, these findings should trigger the design of *in vivo* animal studies on the fluorescence-guided laparoscopic surgery (FGLS) after QD-labeling of CRT-rich solid pancreatic lesions [5]. The advantages of FGLS have already been described by Metildi *et al* [6]; the theranostic advantage of QD-fluorophores as both diagnostic and therapeutic tracers and carriers of immunochemical



**Figure 1** Laser scanning confocal fluorescence microscopy photomicrographs illustrating a pancreatic neuroendocrine tumor before and after exposure to fluorescent gold quantum dots bio-conjugated to anti-calreticulin polyclonal antibodies

tumoricidal agents and their safety for *in vivo* applications in the treatment of pNETs are yet to be developed and proven.

## References

1. Kartalis N, Pozzi Mucelli RM, Sundin A. Recent developments in imaging of pancreatic neuroendocrine tumors. *Ann Gastroenterol* 2015;**28**:193-202.
2. Sheng V, Cheng C, Dong M, et al. Overexpression of calreticulin contributes to the development and progression of pancreatic cancer. *J Cell Physiol* 2014;**229**:887-897.
3. Ghaderi S, Ramesh B, Seifalian AM. Fluorescence nanoparticles 'quantum dots' as drug delivery system and their toxicity: A review. *J Drug Target* 2011;**7**:475-486.
4. Cuenca A, Jiang H, Hochwald S, et al. Emerging implications of nanotechnology on cancer diagnostics and therapeutics. *Cancer* 2006;**107**:459-466.
5. Giorgakis E, Loizidou M, Mavroeidis M, et al. Fluorescence-guided laparoscopic surgery: What if we could label pancreatic cancer with biomarker-conjugated fluorescent quantum nanocrystals? *J Am Coll Surg* 2015;**220**:376-377.
6. Metildi C, Kaushal LS, Luiken G, et al. Advantages of fluorescence-guided laparoscopic surgery of pancreatic cancer labelled with fluorescent anti-carcinoembryonic antigen antibodies in an orthotopic mouse model. *J Am Coll Surg* 2014;**219**:132-141.

<sup>a</sup>Division of Surgery and Interventional Science, University College London (Emmanouil Giorgakis, Bala Ramesh, Marilena Loizidou);

<sup>b</sup>Minimal Access and Hepatobiliary Surgery, King's College Hospital NHS Foundation Trust (Emmanouil Giorgakis), London, UK

Conflict of Interest: None

Correspondence to: Emmanouil Giorgakis MD, Minimal Access Surgery & Hepatobiliary Surgery, King's College Hospital NHS Foundation Trust, Denmark Hill, London, SE5 9RS, United Kingdom, Tel.: +44 020 3299 900, e-mail: emmanouil.giorgakis@nhs.net

Received 13 May 2015; accepted 22 May 2015