

Demonstration of Calreticulin Expression in Hamster Pancreatic Adenocarcinoma with the Use of Fluorescent Gold Quantum Dots

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Abstract. *Background: There is dire need for discovery of novel pancreatic cancer biomarkers and of agents with the potential for simultaneous diagnostic and therapeutic capacity. This study demonstrates calreticulin expression on hamster pancreatic adenocarcinoma via bio-conjugated gold quantum dots (AuQDs). Materials and Methods: Hamster pancreatic adenocarcinoma cells were cultured, fixed and incubated with fluorescent AuQDs, bio-conjugated to anti-calreticulin antibodies. Anti-calreticulin and AuQDs were produced in-house. AuQDs were manufactured to emit in the near-infrared. Cells were further characterized under confocal fluorescence. Results: AuQDs were confirmed to emit in the near-infrared. AuQD bio-conjugation to calreticulin was confirmed via dot-blotting. Upon laser excitation and post-incubation with bio-conjugated AuQDs, pancreatic cancer cell lines emitted fluorescence in near-infrared. Conclusion: Hamster pancreatic cancer cells express calreticulin, which may be labelled with AuQDs. This study demonstrates the application of nanoparticle-based theranostics in pancreatic cancer. Such biomarker-targeting nanosystems are anticipated to play a significant role in the management of pancreatic cancer.*

Pancreatic cancer is a formidable disease, with rising incidence. Patients usually experience no detectable symptoms until the cancer has spread to other organs. Only about 10% of exocrine pancreatic malignancies are contained within the pancreas at

the time of diagnosis and only about half of these prove to be resectable (1). In England and Wales, only 1% of people diagnosed with pancreatic malignancy survive for 10 years (2). Without surgical resection median survival is 2 to 6 months and estimated 5-year survival is 0.4 to 5% (3). Chemotherapy improves pancreatic cancer survival only marginally (4). Surgical resection is the only possible curative treatment; however, only 10 to 15% of patients are candidates for curative resection upon their initial presentation. Moreover, pancreatectomies are associated with considerable morbidity and mortality rates outside specialising centres, even though in high-volume centres these lie between 3% and 5% (5).

There is currently dire need for gaining further insight into pancreatic carcinogenesis in order to identify biomarkers for cystic and solid pancreatic lesions and for resourcing novel ways of tackling the disease. One potential biomarker is calreticulin, a ubiquitous Ca²⁺-binding protein with chaperone activity, which is highly conserved among species. Calreticulin resides in the endoplasmic reticulum. It is also found in the nucleus, suggesting that it may have a role in transcriptional regulation. It has three domains (6), each responsible for a different function, namely calcium binding (7), chaperoning (8), integrin binding and steroid hormone receptor modulation.

Calreticulin expression has been positively correlated with various types of cancer (9), including pancreatic (10). Not only is calreticulin overexpressed in pancreatic cancer, but it also contributes to its progression; it has been shown that its expression is positively correlated with tumour International Union Against Cancer stage and lymphatic metastasis status (11). It has further been demonstrated that calreticulin is expressed on the cell surface in various human cancer types, including bladder, ovarian and brain cancer, conferring worse clinical prognosis (12). However, calreticulin expression on the surface of pancreatobiliary cancerous cells is yet to be proven. Perhaps contrary to the above, calreticulin appears to promote immunogenic cell death by modulating cytotoxic lymphocytic activity (12) and promoting cancerous cell phagocytosis (13).

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Calreticulin seems to be translocated to the cancer cell surface, becoming the dominant pro-phagocytic signal for cancer cell death (12); notably, this latter study did not include pancreatic tissue specimens, hence calreticulin cell-surface expression on dysplastic or cancerous pancreatic cells is yet to be proven.

Quantum dots (QD), or nanocrystals, are semiconductor crystal structures of nanoscale dimensions (2-10 nm). They are made of semiconductor materials that are small enough to exhibit quantum mechanical properties. Their electronic properties are intermediate between those of bulk semiconductors and discrete molecules.

Due to their size, semiconductor QD nanoparticles bear unique optical properties, such as highly tuneable fluorescence, sharp emission spectra, high quantum yield and remarkable photostability (14). Apart from these characteristics, their relatively large surface-to-mass ratio allows them to bind and adsorb compounds, such as antibodies, peptides, probes or drugs. These attributes provide QDs with a unique potential for a huge range of applications, ranging from tagged biomarker development for cancer detection or precancerous screening, to targeted treatments with drug delivery, cancer vaccinations or tissue labelling prior to resection. QDs also have great potential in photodynamic therapy, where they act as either photosensitizers themselves or as carriers. All that being said, QD-based drug delivery has been relatively less investigated, largely due to the potential innate toxicity of QDs. This problem is more prominent with the first-generation QDs, where toxic Cd and Pb were frequently used in QD preparation. These elements are prone to produce toxic products upon photo-oxidation. It is, therefore, necessary to manufacture QDs from less toxic metals in order to facilitate their safe *in vivo* application as theranostic agents. One potentially suitable element is gold (Au).

The aim of this study was twofold: to demonstrate calreticulin expression in hamster pancreatic adenocarcinoma; and to demonstrate the development of gold nanoparticles and their successful bio-conjugation to rabbit polyclonal antibodies to calreticulin (calreticulin pAbs).

Materials and Methods

AuQDs, as well as calreticulin synthetic peptide and anti-calreticulin polyclonal antibodies, were all produced at the laboratory of the Surgery Research Department of the University College of London, at the Royal Free Campus. The AuQDs were manufactured to emit in near infra-red (NIR). The size and pattern of these QDs were further characterised with the use of transmission electron microscopy. Anti-calreticulin pAb specificity was confirmed with competitive inhibition assay. The anti-calreticulin antibodies were bio-conjugated to the AuQDs, which was confirmed by dotblotting.

Syrian golden hamster (GN strain) pancreatic adenocarcinoma cell lines (HaP-T1) originating from Japan (University College London Biobank) were cultured at the tissue culture laboratory at the Surgery Research Department of the University College of London. Cells were cultured in six-well plates, which were then washed with phosphate-buffered saline (PBS). Cells in half of the wells were then incubated

with calreticulin pAb-conjugated AuQDs. The HaP-T1 colonies not exposed to calreticulin pAbs served as controls. Samples of these cell lines were then fixed on slides and viewed under laser scanning confocal microscopy. The images obtained underwent qualitative and quantification analysis, based on the pixel intensity of luminance (ImageJ 2.0.0-rc-9; National Institutes of Health, Bethesda, MD, USA). Continuous digital data obtained were statistically analysed *via* Wilcoxon signed-ranks test (Wilcoxon Signed-Ranks Test Calculator, Social Statistics Web).

AuQD synthesis. QDs used in this project were produced in-house (Cancer Nanotechnology Group/University College London), assembled *via* a simple nanoparticle synthesis route, based on gold as core metal element and mercaptosuccinic acid (MSA) as stabilising and coating agent (Ramesh B, Giorgakis E, Lopez-Davila V, Kamali-Dashtarzheneha A, Loizidou M, unpublished observations). In brief, the method involved using a borate/citrate buffer at 25 mM at pH 6.5. Instead of using sodium tetrahydridoborate as a reducing agent in the formation of QDs, 200 μ m of dimethylformamide was introduced as a reductant into the buffer whilst mixing vigorously. This mixture was then exposed to hydrothermal treatment (autoclave) at 121°C for 25 minutes to give QDs with near infrared (NIR) emission at >800 nm. The water-soluble NIR-emitting QDs obtained were further characterised using spectrophotometric analysis. The size and pattern of these QDs were further confirmed by transmission electron microscopy. These nanocrystals were configured in the form of crystals or clusters, measuring in the range of 2 μ m.

Production of anti-calreticulin synthetic peptide IgG antibodies. The AuQDs produced were bio-conjugated with calreticulin pAbs prior to pancreatic cell calreticulin targeting. This required the generation of anti-serum to human calreticulin. A 15 amino-acid peptide corresponding to calreticulin amino-terminus was synthesised in-house (Ramesh B, Giorgakis E, Lopez-Davila V, Kamali-Dashtarzheneha A, Loizidou M, unpublished observations). This peptide was coupled to a protein carrier. The protein carrier used was Keyhole Limpet haemocyanin (KLH) (Sigma-Aldrich Company Ltd, Poole, Dorset, UK), which was then coupled to the calreticulin peptide with the addition of glutaldehyde. This yielded KLH-conjugated calreticulin peptide which was then used as an antigen for rabbit immunisation. It was injected into New Zealand white rabbits (intramuscular, 1 mg/rabbit) for calreticulin pAb production. Anti-calreticulin IgG was purified by precipitation with ammonium sulphate.

Calreticulin pAb conjugation to AuQDs. Anti-calreticulin produced as described above was then conjugated to AuQD nanoparticles. Fluorescent AuQD/Au/MSA QD solution was diluted with equal volume of cold ethanol and centrifuged at 1.5 g for 30 minutes. The precipitated Au/MSA QDs were vacuum dried to obtain a dry metal complex. The precipitated dried QDs (approximately 1 mg) were re-suspended in 1 ml PBS. The obtained solution of coated QDs (1 ml) was immuno-conjugated to the rabbit calreticulin pAb using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as an acylating agent. EDC was used in combination with *N*-hydroxysuccinimide (NHS). Briefly, 200 ml QD/MSA solution (1 mg/ml) was mixed with 200 ml EDC (1 mg/ml) and 200 ml NHS (1 mg/ml) in PBS for 30 minutes at room temperature before adding 100 ml of calreticulin pAb solution (5 mg/ml) to the mixture and mixing for 1 hour at room temperature. To separate the reagent and unconjugated AuQDs, membrane

centrifugal columns (Centricon Thermo Fisher Scientific, Waltham, MA, USA) with a cut-off of 100 kDa with UV monitoring at 280 nm of the retained samples was used. The purified Au/MSA QDs conjugated to calreticulin pAbs were collected and stored at 4°C until further use.

DotBlot confirmation of calreticulin synthetic peptide conjugation to AuQDs. AuQD–calreticulin pAb binding to calreticulin synthetic peptide was demonstrated by dot-blotting. In brief, 5 µl of 1:1000 dilution of AuQD–calreticulin pAb (1 mg/ml) in water was blotted onto aqueous equilibrated polyvinylidene difluoride (PVDF) membrane (Hybond P; Amersham Pharmacia Biotech, Bucks, UK) and allowed to dry at room temperature. The membrane was then rinsed briefly in PBS with 0.05% Tween 20 (PBS/T; pH 7.4) and incubated overnight at room temperature in 2% bovine serum albumin with 0.005% sodium azide for blocking any residual binding sites on the membrane. This was followed by three further rinses (5 minutes each) with PBS/T prior to application of anti-rabbit monoclonal IgG horse radish peroxidase (HRP)-conjugated Ab (Amersham ECL Rabbit IgG, HRP-linked whole donkey Ab, from GE Healthcare Life Sciences, GE Healthcare UK Limited, Amersham Place, Buckinghamshire, UK) and left to incubate at room temperature for one hour (1:5000 dilution, as recommended by the manufacturer). HRP-conjugated anti-rabbit monoclonal IgG would detect AuQD–calreticulin pAb, validating primary antibody conjugation. Following five further rinsing steps with PBS/T (of 5 minutes each), the polyvinylidene difluoride membrane was developed using SuperSignalR West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) detection of the dot blots. Chemiluminescence was detected by gel documentation instrument (BIO-RAD, Molecular ImagerR Chemic DocTm XRS+, Bio-Rad, Hercules, CA, USA) AuQD fluorescence was also detected by the instrument using ultraviolet (UV) excitation and longpass (LP) filters.

Hamster Syrian pancreatic adenocarcinoma cell line (HaP-T1) culture. This cell line was derived from a Syrian golden hamster (GN strain) pancreatic adenocarcinoma (Sigma Aldrich Co. LLC, Toyama Medical and Pharmaceutical University, Toyama, Japan). The cells were cultured in six well plates in 5% CO₂ Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, penicillin (10,000 units/ml), streptomycin (10,000 µg/ml), 1 mM pyruvate, 2 mM glutamine and 10 mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (Gibco® HEPES, Thermo Fisher Scientific). Cells were seeded at 10⁶ per well and incubated for 24 hours. After that time, the cells were washed with PBS (pH 7.4) and fixed with 4% paraformaldehyde solution for 1 hour at room temperature. Cells were washed three times with PBS. They were incubated with calreticulin pAb-conjugated AuQDs in PBS with 0.6% bovine serum albumin for 1 hour and were then washed three times with PBS. HaP-T1 colonies in the cell culture plate wells not exposed to AuQD–calreticulin pAbs served as controls.

Laser scanning confocal fluorescent microscopy (LSCM). The paraformaldehyde fixed slides of hamster pancreatic cell lines were viewed under an LSCM (Nikon Eclipse TE 300, Nikon, Chiyoda, Tokyo City, Tokyo, Japan). The slides were illuminated with 488 nm laser and filtered with 650/LP barriers. Since the AuQDs used were designed to emit in the near infrared (beyond 800 nm), which is difficult to visualise without an NIR camera, their mapped red blue green (RBG) images were captured in the red pseudo-colour spectral band.

Statistical analysis. The digital photomicrographs of HaP-T1 cell lines were further analysed with the use of FIJI (ImageJ 2.0.0-rc-9, National Institutes of Health). Histograms were developed for all captures taken with the use of LSCM (Nikon TE 300) under excitation with a 488 nm laser beam and passage through 650 nm/LP filter in order to plot the tonal distribution of the respective digital images. The digital data obtained were subjected to statistical interpretation. Specifically, the pixel tone medians derived from the digital images' quantification analysis (ImageJ 2.0.0-rc-9) of HaP-T1 cell plates were compared for testing the hypothesis that the medians of pixel tone between images of AuQD–calreticulin pAb exposed HaP-T1 cell lines vs. HaP-T1 controls were identical. The statistical method used was single-tailed Wilcoxon signed-ranks test (Wilcoxon Signed-Ranks Test Calculator, Social Statistics Web). The level of significance was set to $p < 0.05$.

Results

Dot-blotting. By dot-blot incubating of progressive dilutions of AuQD–calreticulin in the presence of anti-rabbit HRP-conjugated Ab, it was demonstrated that the calreticulin pAb was bound to the AuQDs (Figure 1a). The same blots emitted progressively decreasing fluorescence on excitation with UV which was detected with the use of LP filters (Figure 1b). This decreasing chemiluminescence of the progressive dilutions of AuQD–calreticulin pAb in the presence of HRP-conjugated Abs demonstrated the presence of rabbit anti-calreticulin pAb in the blots. Likewise, the decreasing fluorescence under UV excitation during progressive dilution demonstrated the presence of AuQD fluorophores in the same blots.

LSCM (Figure 2). After He-Ne laser excitation, the HaP-T1 cells that had undergone incubation with AuQD–calreticulin pAbs emitted in the NIR. Fluorescence emission of HaP-T1 cells not incubated with calreticulin pAb-conjugated AuQD excitation under the same wavelength light was only limited to background noise. This significantly increased NIR fluorescence of the cancerous cells incubated with calreticulin pAb and could be only related to the binding of the AuQD–calreticulin pAb fluorophores, indicating the presence of calreticulin on the surface and/or the extracellular milieu of the pancreatic cancerous cells. Perhaps as expected, HaP-T1 fluorescence was uniform, apart from some areas of ground-glass luminescence, probably indicative of more densely-packed cellular material or cellular lysis with resultant higher levels of unbound calreticulin. Note was also made of rounded highly fluorescent areas in the wells that had been treated with conjugated AuQDs, which might be representative of AuQD congregations.

The significantly increased NIR fluorescence of HaP-T1 cell lines after exposure to AuQD–calreticulin pAbs provides evidence of calreticulin pAb binding to the fixed hamster pancreatic adenocarcinoma cells, which could only be possible in the presence of calreticulin in the surface or the extracellular milieu of the cancerous cells.

Quantification of digital images demonstrated that the tone distribution of HaP-T1 cells in incubated with

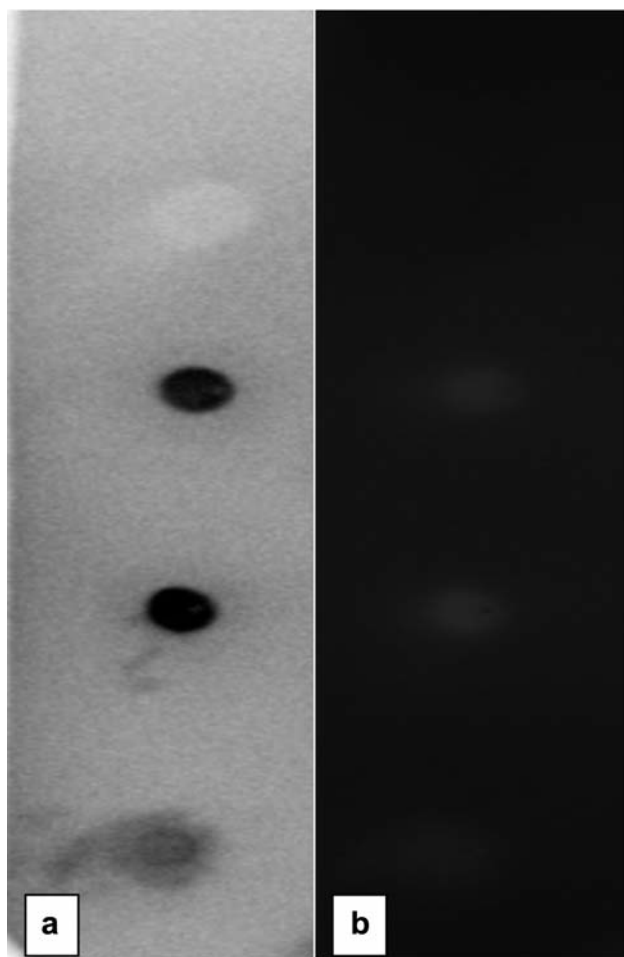


Figure 1. Dot blot of gold quantum dot (AuQD)-calreticulin polyclonal antibodies (pAbs) in the presence of anti-rabbit horseradish peroxidase-conjugated antibody. In progressive dilutions (left to right: 1:10, 1:100, 1:1,000 and 1:10,000), of AuQD-calreticulin pAbs, the blots produced decreasing chemiluminescence (a). Likewise, under UV light, the gelDoc detected decreasing UV fluorescence in the same dilutions, thus demonstrating the presence of the fluorescent AuQDs (b). As described in the Materials and Methods section, the AuQDs used on these experiments had been manufactured to emit in the near infra red.

AuQD-calreticulin pAb was different from that of its paired control. This demonstrated that HaP-T1 retained AuQD-calreticulin pAbs, which further shows that hamster pancreatic adenocarcinoma expresses calreticulin.

The experiments on hamster pancreatic adenocarcinoma cell lines (HaP-T1) lead to the conclusions that: Hamster Syrian pancreas adenocarcinoma can be labelled on fixed tissue sections with AuQDs, bio-conjugated with rabbit pAbs to human calreticulin; hamster Syrian pancreatic adenocarcinoma expresses calreticulin; and calreticulin is expressed in the extracellular milieu of the cancerous cells.

Discussion

Pancreatic cancer survival has not shown much improvement in the last 40 years. It is an almost uniformly fatal cancer, with an incidence rate approaching its mortality rate (1), reflecting the inadequacy of current treatments (16). Chemotherapy improves pancreatic cancer prognosis only marginally (5), leaving surgical resection as the only curative option. Since long-term survival can only be achieved with R0 resection (17), pancreatectomy is currently the cornerstone of pancreatic cancer treatment.

The aim of this study was to demonstrate that calreticulin is expressed on the surface of hamster pancreatic adenocarcinoma. The chosen means for calreticulin targeting were AuQDs. AuQDs, as well as the rabbit pAbs to calreticulin and AuQD-calreticulin pAb bio-conjugation, were all developed in-house by the Cancer Nanotechnology Group (Ramesh B, Giorgakis E, Lopez-Davila V, Kamali-Dashtarzheneha A, Loizidou M, unpublished observations). Cellular surface calreticulin-QD labelling has been demonstrated elsewhere (12); however, in previous studies, the nanocrystals had Cd as core element and the cells were non-cancerous (12). Therefore, this was the first demonstration of calreticulin expression in hamster pancreatic adenocarcinoma.

The justification of semiconductor nanocrystal use as fluorophores for calreticulin molecular labelling was twofold: firstly, because QDs are known favourable alternatives to conventional organic dye fluorophores due to their “intrinsic robustness, high photostability, spectrum tunability, broad absorption spectra and narrow emission peaks” (17), QDs have demonstrated their capability to image fixed cells with “intense brightness, prolonged fluorescence and no photo bleaching” (17). The second reason was because a demonstration of AuQD-calreticulin conjugation in pancreatic malignancy may pave the way for numerous screening, diagnostic and therapeutic applications on pancreatic cancer.

The fixed HaP-T1 hamster pancreatic cancer cell cultures were characterised under confocal fluorescence before and after incubation with AuQD-calreticulin pAb. The fixed cells emitted high NIR fluorescence after incubation with AuQD-calreticulin pAb and 488 nm excitation, demonstrating that they had retained AuQD-calreticulin pAbs. This means that calreticulin was present on the surface of hamster pancreatic adenocarcinoma cells. Note was made of areas with ground-glass appearance with more luminescence, probably indicating the presence of more densely packed cellular material. Note was also made of rounded highly fluorescent areas in the wells treated with conjugated AuQDs, perhaps representing AuQD congregations. The increased NIR fluorescence of HaP-T1 cell lines after exposure to AuQD-calreticulin pAb strongly supports the hypothesis of calreticulin cell surface translocation in hamster pancreatic adenocarcinoma; similar findings have been observed in human cancer cell lines (Ramesh B, Giorgakis E,

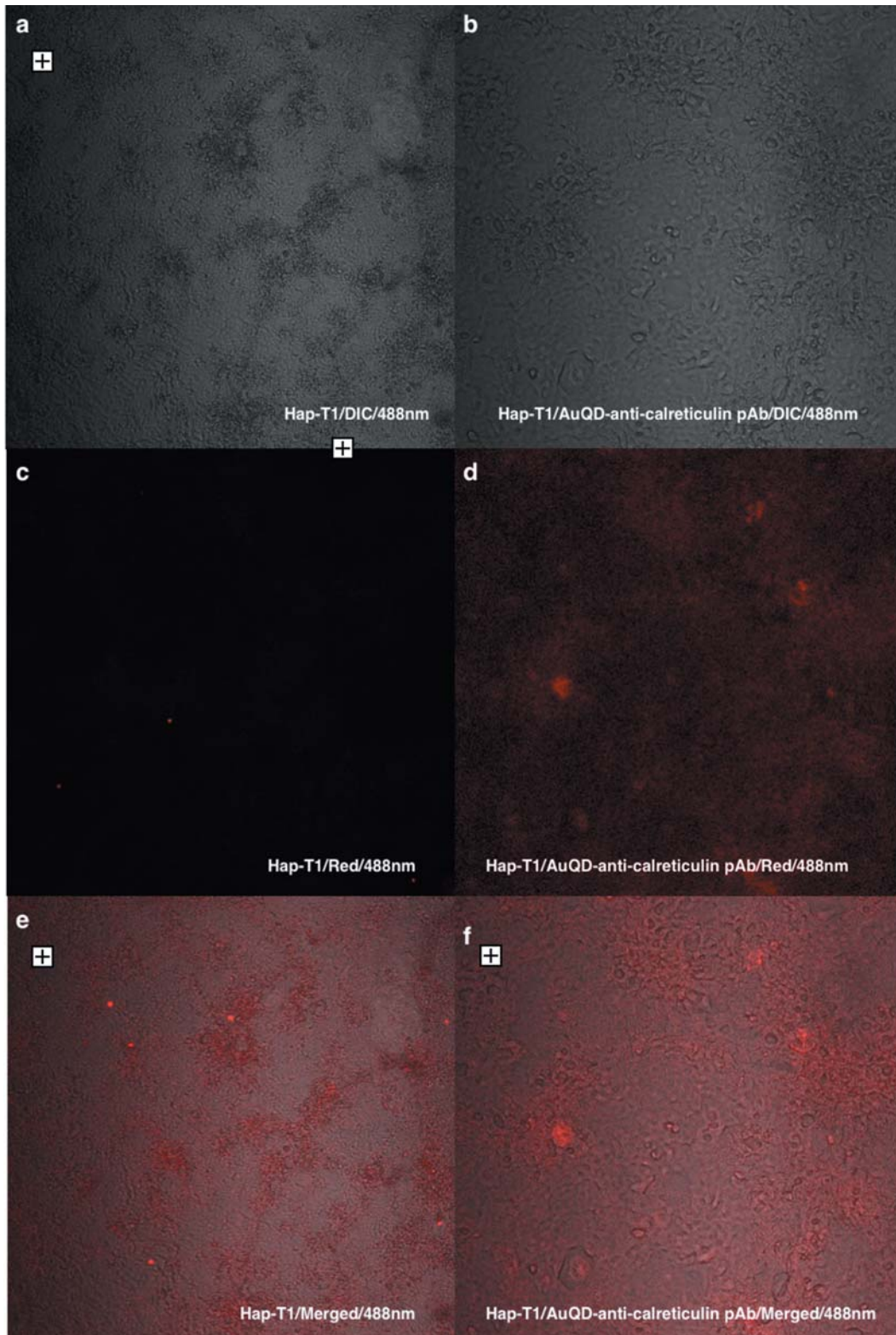


Figure 2. Composite image of digital photomicrographs of hamster pancreatic cancer cells. All images in the right column were taken from wells exposed to gold quantum dot (AuQD)-calreticulin polyclonal antibodies (pAbs). a, b: Differential interference contrast (DIC) captures. d, f: After excitation with 488 nm laser beam and passage through 650/longpass. c, e: DIC/fluorescence overlay. The increased NIR fluorescence of HaP-T1 cells after exposure to AuQD-calreticulin pAbs provides evidence of calreticulin cell-surface expression in hamster pancreatic adenocarcinoma.

Lopez-Davila V, Kamali-Dashtarzheneha A, Loizidou M, unpublished observations).

Digital image data quantification analysis of HaP-T1 demonstrated a shift of the tone plot distribution towards the right hand-side and broader tonal distribution after AuQD-labelling, implying that the images were becoming more fluorescent after AuQD-calreticulin pAb exposure, meaning that the cells had bound or trapped AuQD bio-conjugates, something which would only be feasible in the presence of calreticulin on the surface or in the extracellular milieu of the cancerous cells. AuQD-calreticulin pAb uptake was further demonstrated with digital subtraction of the images, which resulted in sharper mapped RGB images after removal of the background red/NIR fluorescence. Statistical analysis further confirmed the significantly increased fluorescence of the pancreatic cancer cell line in the NIR after incubation with AuQD-calreticulin pAbs ($p < 0.05$).

The finding of calreticulin expression in pancreatic neoplasia and precancerous states has been reported elsewhere (11). Cell-surface calreticulin expression in various types of human cancer cells has already been described (12). According to the Authors' best knowledge, this study provides the first indication of calreticulin cell -surface translocation in pancreatic ductal adenocarcinoma.

Even though calreticulin cell-surface expression with the use of QDs has been demonstrated elsewhere with the use of Cd QDs (14), this is the first demonstration of calreticulin expression in malignant cells with the use of QDs and specifically the first demonstration of calreticulin labelling in hamster tissue with the use of rabbit calreticulin pAbs conjugated to AuQDs. QDs have been used in the past for labelling human pancreatic cancer cells (19). Qian *et al.* had used Cedex/Cods/Zen's QDs, coated with lysine and anti-claudin-4 (pancreatic cancer-specific monoclonal antibody) as a targeting ligand (20). The use of nanocrystals with Au cores poses a lower toxicity risk (Ramesh B, Giorgakis E, Lopez-Davila V, Kamali-Dashtarzheneha A, Loizidou M, unpublished observations). Beyond this, given the known calreticulin expression in various types of human cancer, bio-conjugated AuQDs might find application in targeting various other calreticulin-positive lesions.

The use of QDs for biomedical application arises from their unique physicochemical properties and their ability to serve as scaffold for additional agents, such as targeting ligands and therapeutic drugs. Gold-based nanoparticle agents have further unique characteristics, such as strong surface absorption, stability, biosafety, and ease of modification; for this reason, gold-based nanoparticles have since long been exploited as a candidate material for building up functional agents for both imaging and therapeutic applications. Nonetheless, there is an urgent need to understand the metabolism of AuQDs and address any related toxicity issues (19). A potential disadvantage of using AuQD diagnostic and therapeutic platforms are their high cost of production.

Further *in vivo* studies need to be undertaken in order to demonstrate selective calreticulin overexpression in human pancreatic adenocarcinoma and replicate its *in vivo* targeting with AuQDs. If calreticulin expression on pancreatic cancer is confirmed in humans, AuQD-calreticulin pAbs can find application in the *in vivo* fluorescence imaging of pancreatic calreticulin-positive tumours: AuQD-labelled calreticulin tracers may be injected into patients undergoing diagnostic laparoscopy or pancreatic resection and then be surgically explored under fluorescence or the frozen sections be reviewed under NIR fluorescence in order to assess for metastatic disease, local disease advancement, vascular infiltration or resection margins.

Vohra *et al.* has already used spectral imaging to trace intravenously injected tumour-labelled QDs into mice (19). Intra-operative fluorescence imaging of colorectal and pancreatic cancer with the use of anti-carcinoembryonic (CEA) fluorophore-conjugated Abs has already been demonstrated elsewhere (21). Metildi *et al.* have already demonstrated that fluorescent-guided laparoscopic surgery (FGS) with the use of fluorophore-conjugated chimeric anti-CEA Abs reduced the recurrence rates and increased disease-free survival and cure rates in an orthotopic mouse model of pancreatic cancer (22). FGS appears to be superior in identifying metastatic disease if compared to conventional laparoscopic surgery (23). Our study demonstrated that calreticulin can be targeted with fluorophore-conjugated calreticulin pAbs in fixed hamster pancreatic cancerous cell lines. The above findings in conjunction indicate that fluorophore-conjugated calreticulin pAbs can be employed as an alternative GFS navigation tool, with AuQDs as the conjugated fluorophore. The demonstrated superiority of FGS in revealing occult metastatic deposits, in conjunction with the overall dominance of QDs in terms of fluorescence emission robustness, photostability and spectrum tunability indicate that FGS with the use of QDs might be one of the ways forward in laparoscopic hepatobiliary and pancreatic surgery, specifically in staging laparoscopy. FGS with the use of AuQD fluorophores is expected to reduce the rate of false-negative staging laparoscopies, thus sparing patients from the sequelae of a laparotomy for inoperable disease (24).

Apart from their utility as a higher fidelity surgical navigation tool, AuQD-labelled calreticulin tracers could also become nanoscale platforms for the delivery of anticancer agents or for thermal ablation of cancerous cells (25, 26).

Conclusion

This project has demonstrated that calreticulin is expressed and may be labelled on fixed cell lines on hamster pancreas ductal adenocarcinoma, indicating calreticulin expression in hamster pancreatic adenocarcinoma; this is in agreement with the demonstration of calreticulin expression on human cancer cell lines. It further demonstrated that AuQDs can be bio-

conjugated to polyclonal antibodies to calreticulin, induced in rabbit using a human calreticulin peptide analogue.

In theory, AuQDs can deliver therapeutic agents to a diseased area and their unique physical properties be exploited to improve diagnosis and monitor therapeutic response, since AuQD nano-platforms can be relatively easily manipulated and loaded with appropriate therapeutic agents. Nonetheless, despite the progress in nanoparticle theranostics, there are disadvantages to be overcome, such as QD toxicity and cost to name a few.

The findings of this study may open the gates for further investigation of calreticulin expression in human pancreatic cancer and on the application of QD-based theranostics in its timely detection and treatment. Despite its promise, our evidence-based insight is still far from adequate and our preliminary findings should trigger further investigation in this challenging yet highly inviting field.

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