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Detecting Cell Surface Expression of Calreticulin in Pancreatic Neuroendocrine Tumors Using a Novel [68Ga]-Radiolabeled Peptide

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BACKGROUND

Current theragnostic techniques for pancreatic neuroendocrine tumors (pNETs) exploit the overexpression of somatostatin receptors (SSTRs) on the cell surface. However, approximately 25% of low-grade and most high-grade pNETs do not express SSTRs, requiring alternative theranostics. Calreticulin (CALR) is a protein linked to reticular calcium homeostasis and immunogenic cell death. Upon sufficient cellular insult, CALR translocates from the endoplasmic reticulum (ER) to the plasma membrane. This then promotes phagocytosis of the damaged cell, facilitating an immune response and potentially serving as a biomarker. Herein, we aimed to characterize CALR expression in pNETs and to induce CALR surface translocation in pNETs and to detect surface CALR using a novel radiolabeled peptide.

METHODS

Tissue microarrays of human pNETs and normal islets were immunohistochemically stained and CALR expression measured via H-scoring by a pathologist. Surface translocation of CALR was detected by flow cytometry in pNET cells (BON, QGP) treated with either dantrolene or doxorubicin. For peptide radiolabeling, the radionuclide-binding chelator 'DOTA' was covalently linked to a CALR-specific peptide 'KLGFFKR'. Then, the peptide [DOTA-Bn-SCN-$_{\beta}$AD$_{\beta}$A$_{\beta}$AKLGFFKR] was labeled with ^{68}Ga. Samples were analyzed on HPLC with an average radiolabeling efficiency of 93%. For in vitro radiopeptide uptake studies, pNET cells were treated with dantrolene for CALR induction and then incubated with 1 µM [^{68}Ga]DOTA-Bn-SCN-$_{\beta}$AD$_{\beta}$A$_{\beta}$AKLGFFKR for 1 hour. For the in vivo biodistribution study, BALB/c mice (n=4) were injected with ~3 MBq (5 µg) of radiopeptide for 1 hour.

RESULTS

Mean H-score of CALR expression was higher in pNETs (241, n=51) compared to normal islets (53, n=17; $p<0.001$). We found that surface CALR can be significantly induced in pNET cells with the ryanodine receptor antagonist dantrolene or the anthracycline doxorubicin. Our novel [^{68}Ga]-CALR peptide showed significantly higher binding in pNET cells when surface CALR was induced by dantrolene (n=3, $p = 0.01$). We also performed an initial biodistribution study using non-tumor bearing BALB/c mice and saw rapid clearance was through the kidneys with no significant uptake in vital organs (n=4).

CONCLUSIONS

CALR can be translocated to the cell surface in pNETs cells, where it can then be detected by a radiopeptide PET imaging agent. The utilization of an alternative pNET cell surface marker, such as CALR, as a therapeutic target could create new treatment options for the subset of patients with pNETs that have low basal expression of SSTRs.

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