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Surface calreticulin induction by doxorubicin in a patient derived xenograft model of pancreatic neuroendocrine carcinoma

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BACKGROUND

Pancreatic neuroendocrine neoplasms are categorized as either well-differentiated (PNETs) or poorly differentiated (PNECs). PNETs have lower proliferative rates and often overexpress somatostatin receptors (SSTRs), enabling SSTR-targeted theragnostics. In contrast, PNECs are highly proliferative, often do not express SSTRs, and the 5-year patient mortality rate is over 50%. Therefore, new treatment options for patients with PNECs and SSTR-negative PNETs are needed. Calreticulin (CALR) is a protein linked to endoplasmic reticular (ER) calcium homeostasis and immunogenic cell death. Upon sufficient cellular insult, CALR can translocate from the ER to the cell surface. Surface CALR can be targeted with theragnostic agents for tumor imaging and potential treatment. Herein, we hypothesized that doxorubicin, a common agent to trigger CALR surface translocation, could induce surface CALR in a PNEC patient-derived xenograft model (PDX).

METHODS

The PNEC PDX used in this study was derived from a surgically resected PNEC. Multiple generations were histologically validated by a surgical pathologist using H&E, chromogranin, synaptophysin, and Ki-67 staining. Tumors were grown subcutaneously in mice following an approved IACUC protocol, and at approximately 250mm^3 tumor volume, mice were randomized and treated with either saline, 0.22mg/kg intratumoral doxorubicin, or 10mg/kg intraperitoneal doxorubicin (n=6/group). Tumors were harvested 24h after intratumoral or 48h after intraperitoneal treatment. Tumors were halved and analyzed for surface CALR by immunofluorescence and flow cytometry. A fixable live/dead stain was included for flow. Surface CALR detected by immunofluorescence was quantified in FIJI and flow cytometry data was analyzed in FlowJo. Statistics were calculated in SPSS using an ANOVA with Tukey post hoc test.

RESULTS

Immunofluorescence revealed that tumors treated with intratumoral doxorubicin had a background-subtracted mean raw integrated density for surface CALR expression of 8.7 x 10^9, versus 7.2 x 10^9 for both saline and intraperitoneal doxorubicin ($p < 0.001$). In parallel, flow cytometry analysis showed tumors treated with intratumoral doxorubicin had 26.5% of live cells (49.5% total cells) expressing surface CALR, compared to 7.9% of live cells (17.4% total cells) expressing surface CALR in the saline group ($p = 0.032$). Tumors treated with intraperitoneal doxorubicin had 9.6% live cells (28.8% total cells) with surface CALR.

CONCLUSIONS

We found that surface CALR expression was increased in PNEC PDXs 24h after administration of intratumoral doxorubicin compared to saline injected tumors. Systemically administered doxorubicin did not significantly increase surface CALR expression, which suggests other delivery methods or inductions agents may be preferred to induce surface CALR prior to theragnostic targeting.

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