Irreversible Electroporation (IRE): Preclinical Assessment

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Background

- Irreversible electroporation (IRE) causes irreversible permeabilization of the cell membrane via **short high-voltage** electric pulses.
- IRE offers a promising treatment modality for local ablation of pancreatic ductal adenocarcinoma (PDAC).
- We aim to assess preclinical effects IRE on different types of cells *in vitro* and macrophage infiltration *in vivo*.

Methods

IRE-treated Cells: KRAS* PDAC cells, murine RAW264.7 macrophages, and murine bone marrow derived macrophages (BMDM) were used in following assays.

Assays: MTS assay assessed cell proliferation following IRE. Transwell migration assessed if IRE treated KRAS* cells affected macrophage migration (Fig. 1)

Immunofluorescence (IF) staining: IF staining assessed the density of CD169+ macrophages on KRAS* tumor tissues from non-treated and IRE treated mice.

Liver metastasis model: PH252 cells (a variant of KRAS* PDAC cells) were inoculated into the spleen of mice to develop a liver metastasis model of PDAC. Tumor growth was monitored by T2-weighted MRI.

Statistics: Significance was determined by one-way ANOVA with $p \leq 0.05$ considered statistically significant.

Results

- KRAS cells were more sensitive to IRE than macrophages (BMDM and RAW264.7).
- IRE treated KRAS* tumor cells promoted macrophage migration. This was confirmed in *in vivo* experiment, which showed increased tumor infiltration of CD169+ macrophages 24h after IRE.
- PH252 PDAC tumors injected into the spleen metastasized to the liver.

Conclusion

- IRE decreased cell proliferation of tumor cells and influenced macrophage migration. Because of IRE's effects on macrophages, further studies on the impact of IRE on presentation of tumor associated antigens are warranted.

Future Directions

- Test IRE effects at other voltages and with other cell types
- Refine tumor model by injecting fewer cells or injecting directly to pancreas
- Characterize the effects of local IRE treatment on liver metastasis

In vitro methods

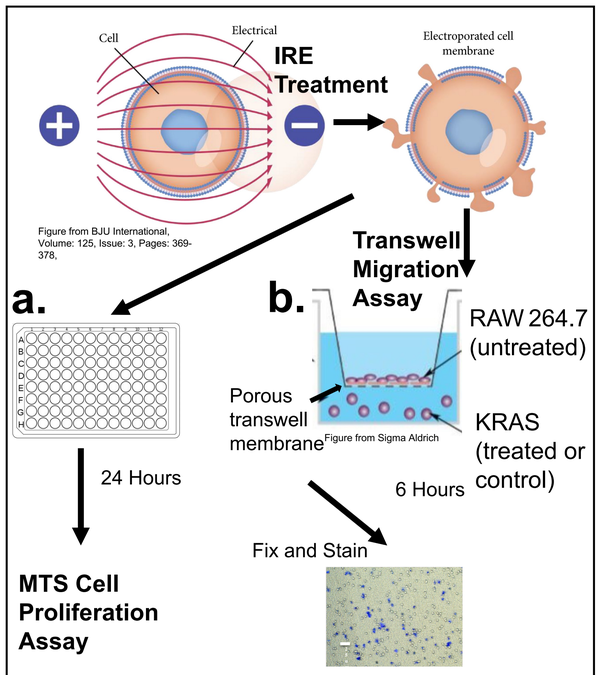

Fig. 1 *in vitro* methods. IRE treat KRAS cells, RAW264.7 macrophages, or Bone Marrow Derived Macrophages (BMDM) with 20 pulses, 0.1 ms long, 1 second between pulses, at 300 or 400 V.

a. Plate cells to 96-well plate and incubate for 24 hours. Then do MTS cell proliferation assay to determine cell concentration.
b. Place macrophages on top of membrane and KRAS tumor cells below. Incubate 6 hours for migration before fixation and staining

Cell Proliferation

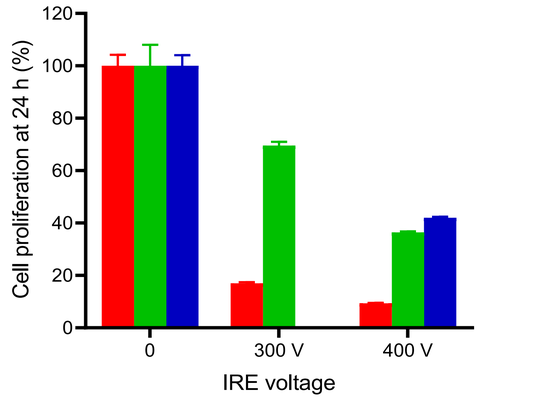

Fig. 2 Cell Proliferation after IRE treatment 24h. KRAS, RAW264.7, and Bone Marrow Derived Macrophages (BMDM) were treated with IRE at 0, 300, or 400 V. Cell concentration was calculated using MTS proliferation assay and is displayed as percentage of control proliferation.

Macrophage Migration

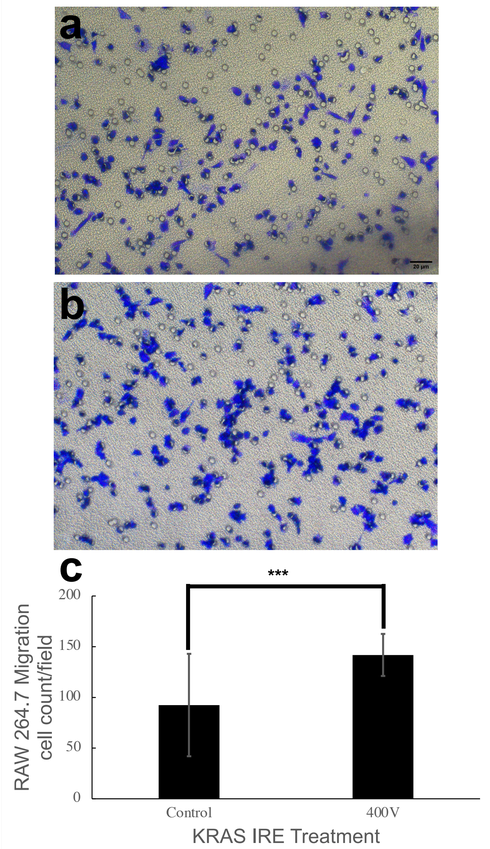

Fig. 3. Assessing impact of IRE treated KRAS cells on macrophage migration. **a,b** Representative images of RAW264.7 migration following 6 hours with control (a) or 400V (b) IRE treated KRAS cells. **c** Bar graph of means and standard deviations of number of cells migrated per field of view. *** $p<0.001$ (one-way ANOVA)

Macrophage IF Staining

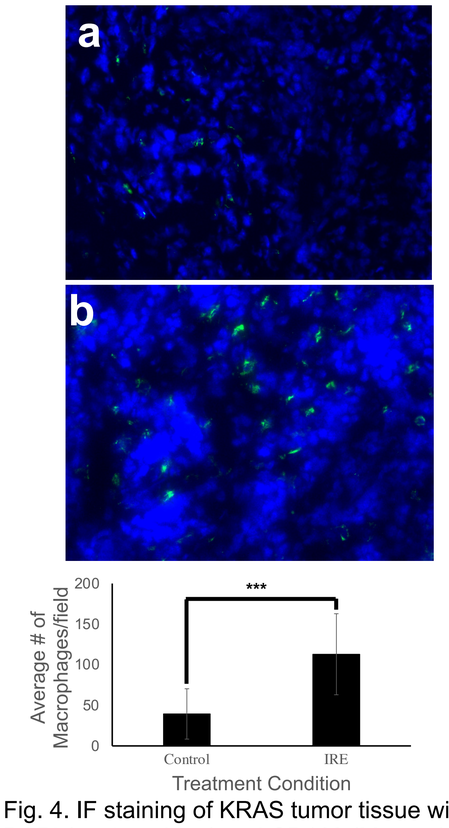

Fig. 4. IF staining of KRAS tumor tissue with DAPI (nuclear, blue) and CD169 (Macrophages, green). **a,b** control and IRE treated tumors, respectively. **c** Bar graph of number of macrophages counted per field of view *** $p<0.0001$ (one-way ANOVA)

Liver metastasis model

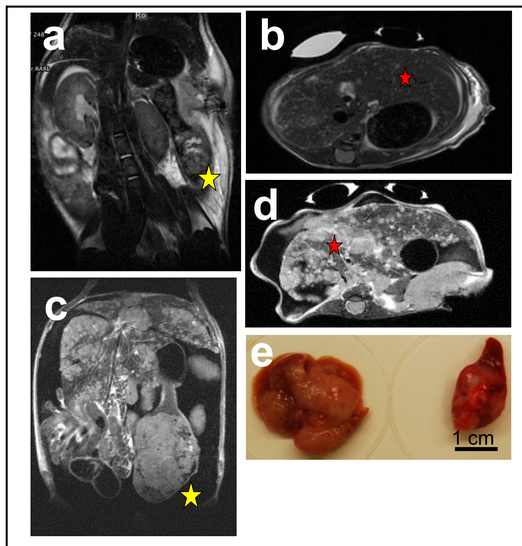

Fig. 5. 2 million cell injection of PH252 PDC into spleen with liver metastasis. **a,c** Coronal view at 1 and 2 weeks, respectively. **b,d** axial view at 1 and 2 weeks, respectively. **e** Liver (left) and spleen (right) of mouse following death 2.5 weeks after injection. Stars placed to bottom right of tumor

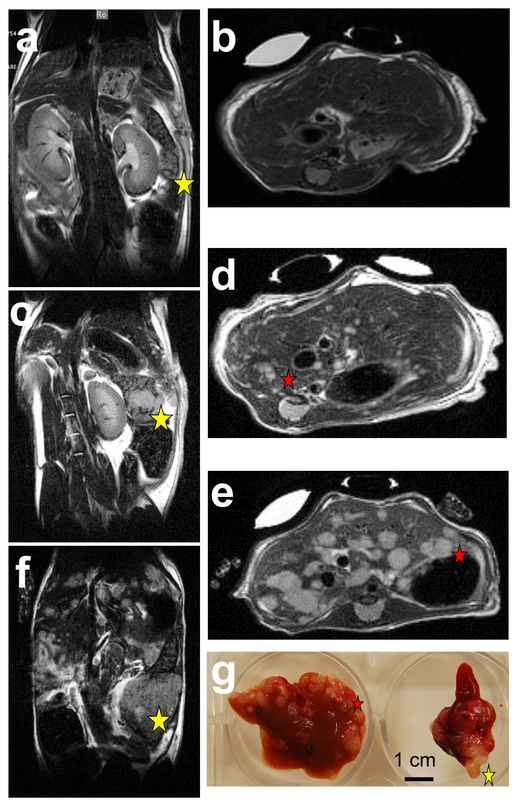

Fig. 6. 0.5 million cell injection of PH252 PDC into spleen with liver metastasis. **a,c,e** Coronal view at 1, 2, and 3 weeks, respectively. **b,d,f** axial view at 1, 2, and 3 weeks, respectively. **g** Liver (left) and spleen (right) of mouse following death 3.5 weeks after injection. Stars placed to bottom right of tumor

References

1. Zhao J, et al. Nature Commun 10:article number: 899 (p1-14), 2/2019
2. Ouyang H. et al. Pancreas 40(1): 120-125 .1/2011

Acknowledgements

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