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Myoferlin targeting triggers mitophagy and primes ferroptosis in pancreatic cancer cells

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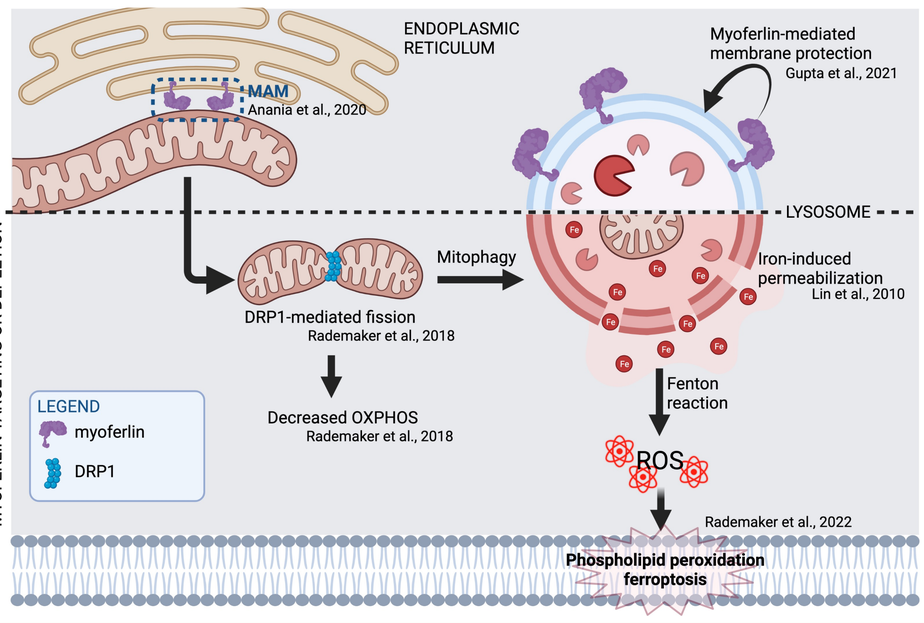
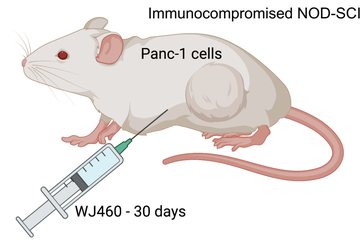


Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related deaths worldwide and it is estimated that PDAC will become the second leading cause of cancer-related death by 2030. Myoferlin is a protein implicated in membrane fusion and in receptor recycling. It allows the formation of vesicles for the trafficking in cells. In PDAC, myoferlin is overexpressed in high grades in comparison to low grades. We used an innovative small compound **(WJ460)** to target myoferlin in PDAC cell lines and reported the triggering of an iron-dependent cell death, namely ferroptosis, considered as an alternative to apoptosis in cancer cells.

In vitro

	HPNE	Panc-1	BxPC-3	PaTu8988T	MiaPaCa-2
Source	Normal epithelium	Primary	Primary	Liver	Primary
KRAS	WT	G12D	WT	G12V	G12C
Daemen's classification	-	LIPOGENIC	LIPOGENIC	GLYCOLYTIC	GLYCOLYTIC
Collisson's classification	-	QM-PDA	QM-PDA	QM-PDA	QM-PDA

In vivo



1 Pharmacological targeting of myoferlin with WJ460 induces the same hallmarks of myoferlin gene silencing

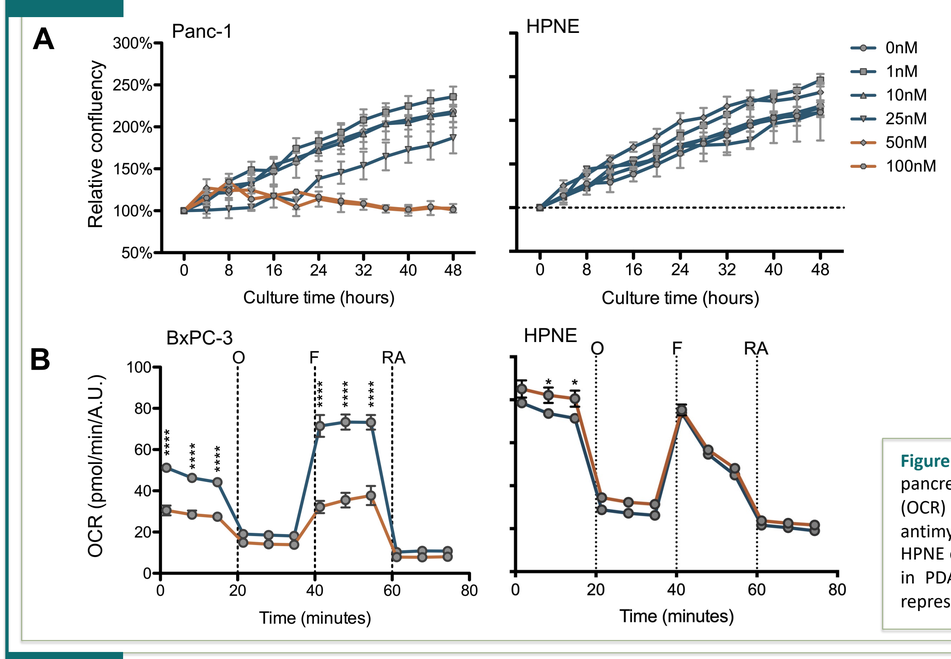


Figure 1: **(A)** Cell growth assessment during treatment of PDAC cell line (Panc-1) and normal pancreatic cell line (HPNE) with WJ460 ranging from 1 to 100 nM. **(B)** Kinetic oxygen consumption rate (OCR) in PDAC cell line (BxPC-3) and HPNE in response to oligomycin (O), FCCP (F), rotenone and antimycin A (RA). **(C)** Cytosolic reactive oxygen species (ROS) accumulation in PDAC cell lines and HPNE cell line treated for 24h with 50 nM of WJ460. **(D)** Visualisation of mitochondrial network shape in PDAC cell line (Patu8988T) and HPNE after 50 nM WJ460 treatment during 24h. Scale bars represent 10 µm (PaTu8988T) or 7.5 µm (HPNE).

****P<0.0001, ***P<0.001, **P<0.01, *P<0.05

2 WJ460 induces mitophagy in pancreatic cancer cells

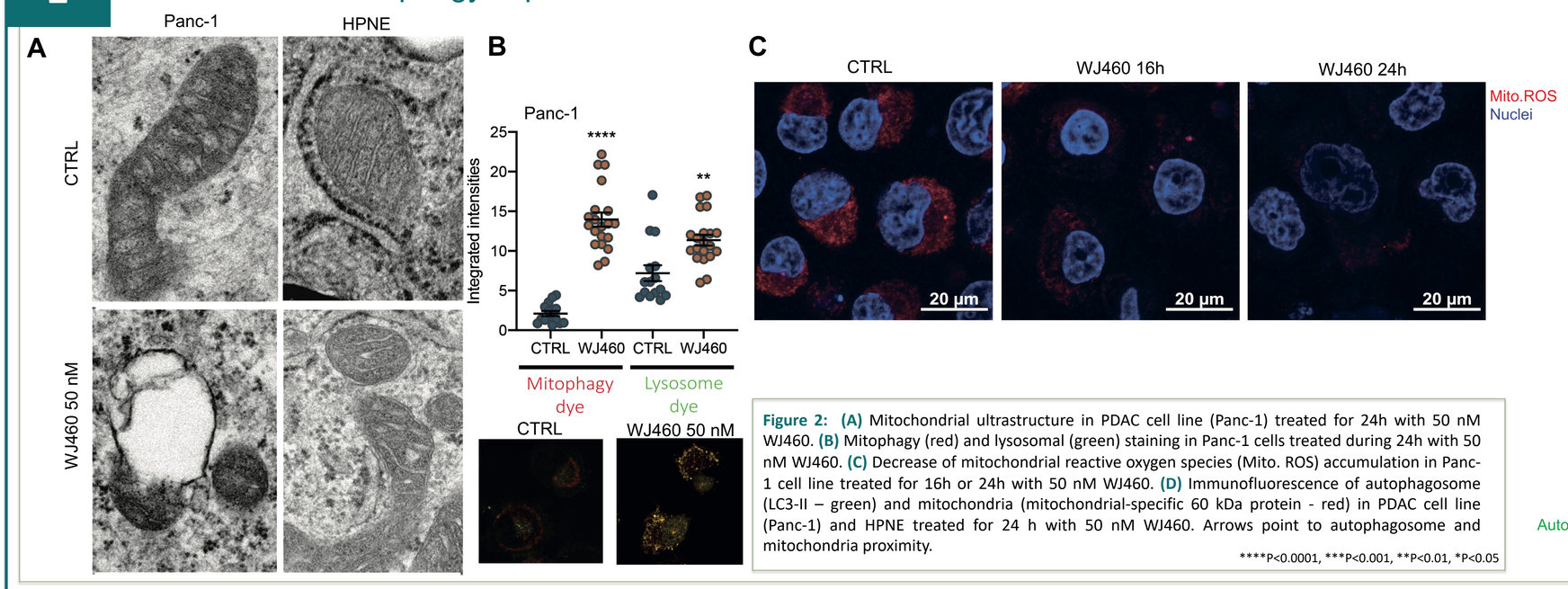


Figure 2: **(A)** Mitochondrial ultrastructure in PDAC cell line (Panc-1) treated for 24h with 50 nM WJ460. **(B)** Mitophagy (red) and lysosomal (green) staining in Panc-1 cells treated during 24h with 50 nM WJ460. **(C)** Decrease of mitochondrial reactive oxygen species (Mito. ROS) accumulation in Panc-1 cell line treated for 16h or 24h with 50 nM WJ460. **(D)** Immunofluorescence of autophagosome (LC3-II – green) and mitochondria (mitochondrial-specific 60 kDa protein - red) in PDAC cell line (Panc-1) and HPNE treated for 24 h with 50 nM WJ460. Arrows point to autophagosome and mitochondria proximity.

****P<0.0001, ***P<0.001, **P<0.01, *P<0.05

3 WJ460 triggers an iron-dependent cell death

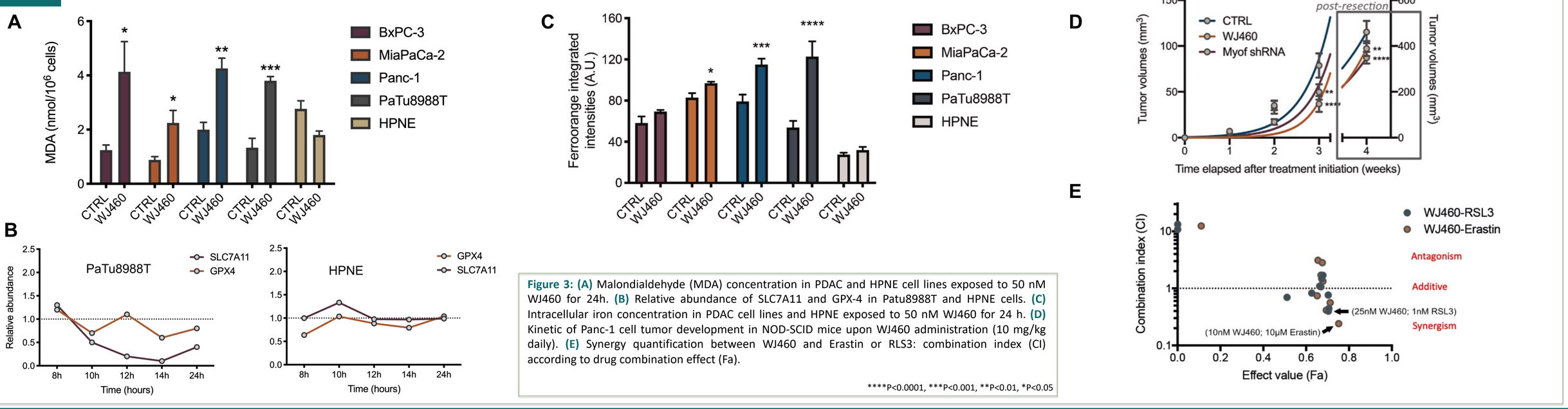


Figure 3: **(A)** Malondialdehyde (MDA) concentration in PDAC and HPNE cell lines exposed to 50 nM WJ460 for 24h. **(B)** Relative abundance of SLC7A11 and GPX-4 in Patu8988T and HPNE cells. **(C)** Intracellular iron concentration in PDAC cell lines and HPNE exposed to 50 nM WJ460 for 24 h. **(D)** Kinetic of Panc-1 cell tumor development in NOD-SCID mice upon WJ460 administration (10 mg/kg daily). **(E)** Synergy quantification between WJ460 and Erastin or RLS3: combination index (CI) according to drug combination effect (Fa).

****P<0.0001, ***P<0.001, **P<0.01, *P<0.05

The mortality rate of PDAC is currently almost equal to its incidence and its 5-year survival rate is the lowest among all cancers. In this study, we described that WJ460, a small compound binding to myoferlin, induced the same main biological effects than myoferlin gene silencing in several PDAC cell lines. Important iron pool is naturally present within tumors. Interestingly, erastin and RSL3, two ferroptosis inducers, were reported to be particularly efficient in KRAS-mutated cancer cells. More than 90% of PDACs carry mutated KRAS alleles, as such, ferroptosis has been proposed as an alternative to treat patients with KRAS mutation. Our results demonstrate that myoferlin targeting induces an iron-dependent cell death in PDAC cell lines.

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