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Shandell, Mia Ann, Lawrence, Samantha, Brackenbury, Will et al. (2019) Ionic modulation of immune checkpoint proteins. In: Physiology 2019, 07-10 Jul 2019, Aberdeen Conference Centre.

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Ionic modulation of immune checkpoint proteins

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Abstract

Despite extensive basic and clinical research on immune checkpoint regulatory pathways, little is known about the effects of the ionic tumour microenvironment on immune checkpoint expression and function.

We screened effects of ion channel modulating compounds on IDO1 activity. Here, we describe a mechanistic link between Na^+/K^+ ATPase inhibition by cardiac glycosides and activity of indoleamine-2',3'-dioxygenase (IDO1), a well-characterized immune checkpoint.

IDO1 catalyses the rate-limiting step of tryptophan catabolism and inhibits the immune response to the tumour by local depletion of tryptophan, an amino acid essential for anabolic functions in cancer and T cells.

Methods

IDO1 activity is measured using a kynurenine assay.

Day 1
Seed cells
Treat with compound

Day 2
Stimulate with 25 ng/mL TNF-α and 1000 U/mL IFN-γ

Day 3
Kynurenine, Alamar Blue assays

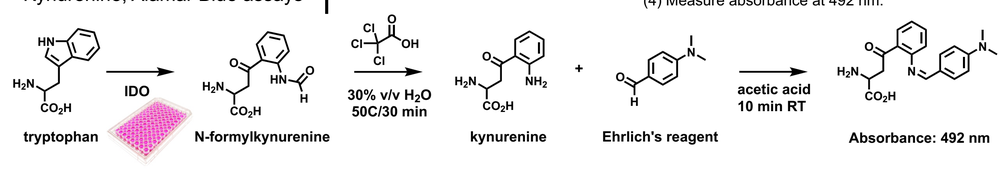
Alamar Blue assay

(after removing supernatant in step (1) Add 10 uL Alamar Blue to cells in tissue culture plate. Incubate 4h 37C/5% CO2. Measure absorbance at 570 nm, 600 nm.

Kynurenine assay of IDO1 activity

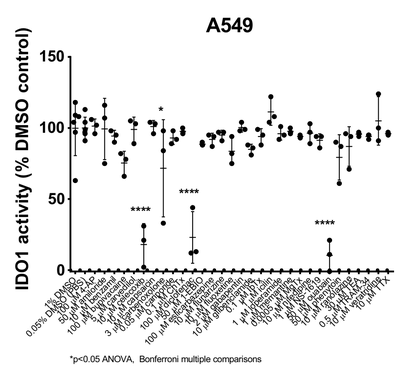
(1) Transfer 150 µL supernatant to a round-bottom plate.
+10 µL 30% v/v trichloroacetic acid.
50°C/30 min

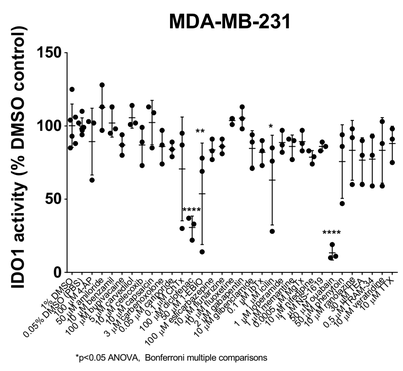
(2) Centrifuge 10 min/3000 rpm/room temperature.
Transfer 100 µL to new plate.
(3) +100 µL 1.2% w/v Ehrlich's reagent in acetic acid. Room temperature/10 min
(4) Measure absorbance at 492 nm.



Ion channel drug screen of IDO1 activity

Drug/toxin	Target(s)	Effect
4-aminopyridine	K_v K^+ channels	Inhibitor
Amiloride	ASIC channel	Inhibitor
Benzamil	Na^+/Ca^{2+} exchanger, ENaC, TRPP3, TRPA1 channels	Inhibitor
Bupivacaine	Na_v, K2P channels	Inhibitor
Carvedilol	$K_v1.5$, $K_{ir}2.3$ K^+ channels, adrenoceptors	Inhibitor
Celecoxib	L-type Ca^{2+} and K_v7 channels	both
Diclofenac (sodium)	K_v7 channels	Activator
Eslicarbazepine acetate	VGSC	Inhibitor
Flunarizine dihydrochloride	L-type and T-type Ca^{2+} channels	Inhibitor
Fluoxetine hydrochloride	$K_v4.3$ channels	Inhibitor
Iberiotoxin	$K_{Ca}1.1$ K^+ channels	Inhibitor
Memantine hydrochloride	NMDA receptor	Inhibitor
Nifedipine	L-type Ca^{2+} channels	Inhibitor
NS-1619	$K_{Ca}1.1$ K^+ channels	Activator
Ouabain	Na^+/K^+ ATPase	Inhibitor
Phenytoin	VGSC, HERG	Inhibitor
Tetraethyl-ammonium chloride	K^+ channels - all	Inhibitor
Tetrodotoxin	VGSC	Inhibitor
TRAM-34	$K_{Ca}3.1$ K^+ channels	Inhibitor
Veratridine	VGSC	Activator
1-EBIO	$K_{Ca}3.1$, $K_{Ca}2$ channels	Activator
Capsaicin	TRPV1 channels	Inhibitor
Carbenoxolone	Pannexin-1 (Gap junction channel)	Inhibitor
Cariporide	NHE1	Inhibitor
Charybdotoxin	$K_{Ca}1.1$, $K_v1.2$, $K_v1.3$ K^+ channels	Inhibitor
Gabapentin	VGCC / alpha-2-delta	Inhibitor
Glibenclamide	TRPA1, KATP channels	Inhibitor
Icilin	TRPM8 channel	Activator
Loperamide hydrochloride	-type, T-type Ca^{2+}, HCN channels, NMDA, μ-opioid receptor	Inhibitor
Margatoxin	$K_v1.3$, $K_v1.6$	Inhibitor
Ranolazine	VGSC	Inhibitor

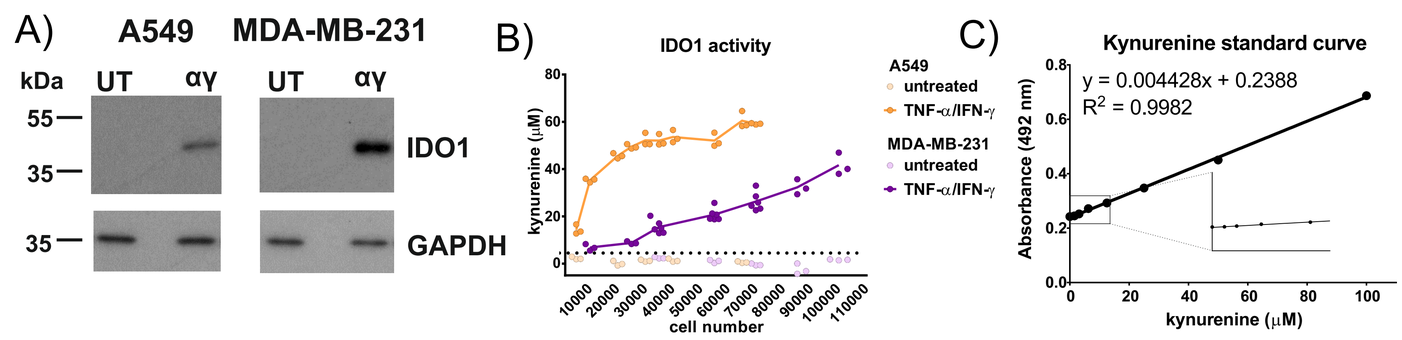




A library of 31 compounds targeting ion channels or pumps was screened in the kynurenine assay measuring IDO1 activity in TNF-α/IFN-γ stimulated lung cancer (A549) and breast cancer (MDA-MB-231) cells. Points (right panel) represent technical replicates and data are normalized to their respective DMSO control, 0.05% or 1% DMSO in PBS.

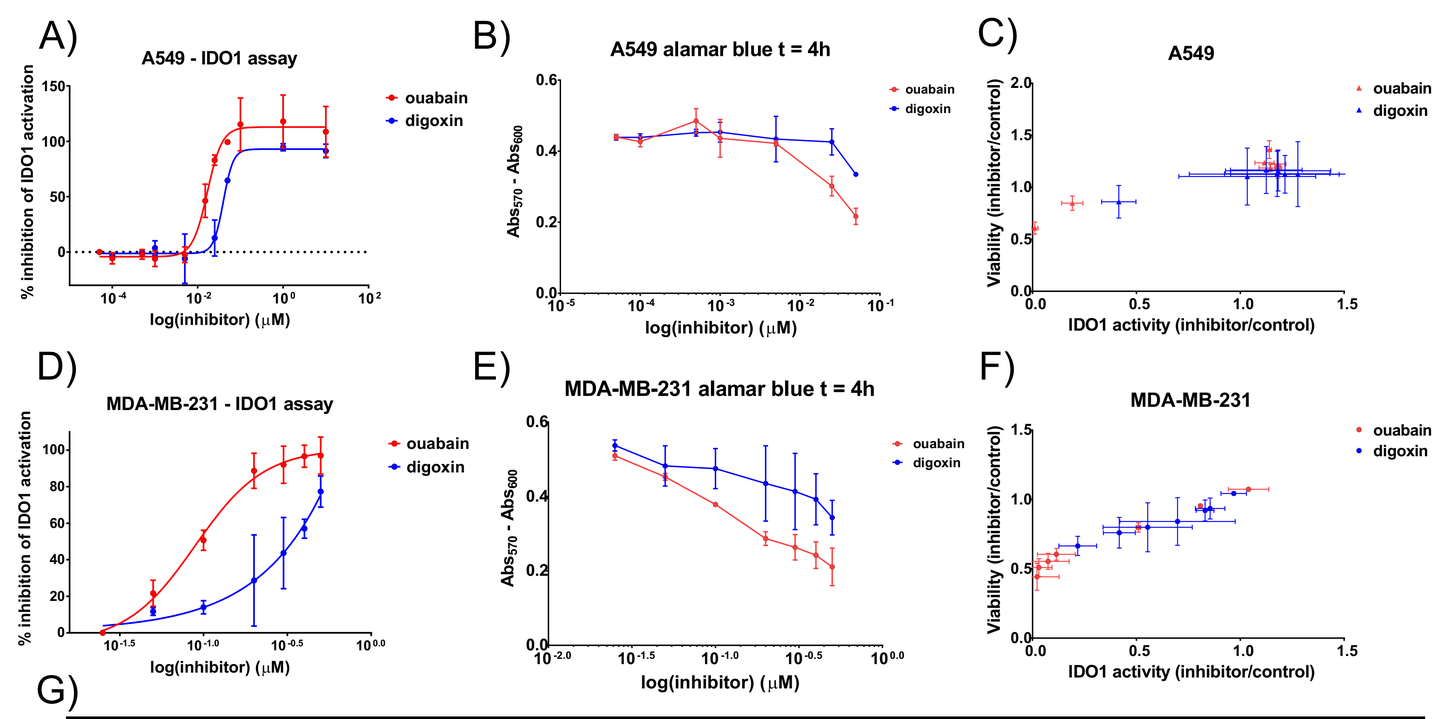
Results

TNF-α/IFN-γ stimulates IDO1 expression in lung cancer A549 and breast cancer MDA-MB-231 cells.



A) Western blot of IDO1 in A549 and MDA-MB-231 cells +/- 25 ng/mL TNF-α/1000 U/mL IFN-γ stimulation. B) IDO1 assay cell titration for A549 and MDA-MB-231 cells. Used 10,000 A549/well and 50,000 MDA-MB-231/well as seeding density in all following experiments. C) Representative kynurenine absorbance standard curve calculated from kynurenine standards in 0.5M HCl.

Cardiac glycosides ouabain and digoxin inhibit kynurenine production in A549 and MDA-MB-231 cancer cells with a modest impact on cell survival.

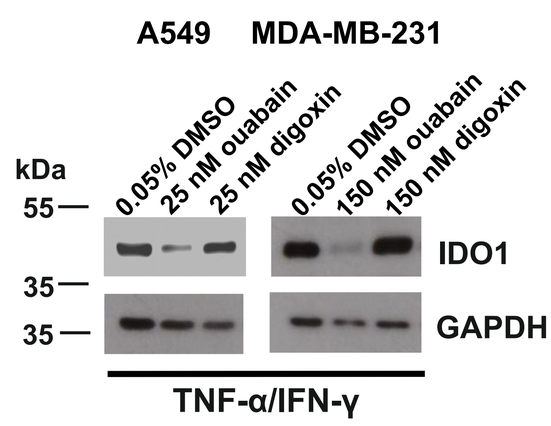


G)

IDO1 activity IC50	A549	MDA-MB-231
ouabain	17 nM	89 nM
digoxin	40 nM	~164 nM

A) Dose-response curves for ouabain and digoxin treatment of TNF-α/IFN-γ stimulated A549 cells. B) Alamar Blue survival assay after 4h incubation with reagent. C) Comparison of IDO1 activity vs. viability as measured by Alamar Blue in A549 cells. D) Dose-response curves for ouabain and digoxin treatment of TNF-α/IFN-γ stimulated MDA-MB-231 cells. E) Alamar Blue survival assay after 4h incubation with reagent. F) Comparison of IDO1 activity vs. viability as measured by Alamar Blue in MDA-MB-231 cells. G) Calculated IDO1 activity IC50s from fitting the dose-response curves by non-linear least squares regression.

Ouabain, but not digoxin, downregulates IDO1 expression in A549 and MDA-MB-231 cancer cells.



Western blot of IDO1 expression in TNF-α/IFN-γ stimulated cells treated with cardiac glycosides. A549 cells were treated with 0.05% DMSO, 25 nM ouabain, or 25 nM digoxin (left panel). MDA-MB-231 cells were treated with 0.05% DMSO, 150 nM ouabain, or 150 nM digoxin (right panel).

Cardiac glycosides decrease activity of immune checkpoint protein IDO1 in cancer cells.

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