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Member of

MultiDrug Resistance (MDR) proteins: active protective system, source of cell longevity

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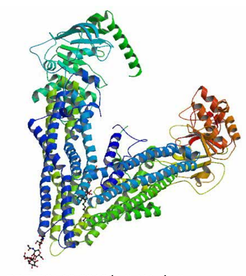
INTRODUCTION

MDR (MultiDrug Resistance) proteins belong to the family of membrane associated transporters. The best known family member is MDR1, also named P-glycoprotein1 (P-gp1) or ATP-Binding Cassette, sub-family B, member 1 (ABCB1).

This transmembrane protein is an active efflux transport system: ATP is hydrolyzed to drive a wide range of diverse substrates from the intracellular cytoplasmic space to the extracellular space. This causes a lower concentration of various endogenous toxic elements, reducing their deleterious effect and resulting in an increase of cell viability.

Key words :
Multidrug Resistance Proteins; P-glycoprotein1; ABCB1; senescence

Some details about MDR:



PDB DOI:10.2210/pdb4f4c/pdb

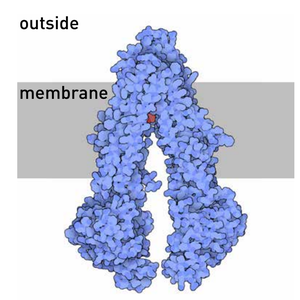
MDR1 is implicated in the protection of the cells against apoptotic cell death.
On bacteria, this process is responsible for antibiotic resistance. On yeast, MDR proteins are associated with cellular metabolism, detoxification and stress response.
MDR are linked with SIRT1 pathway, and the number of cell divisions is correlated with MDR proteins quantity and functionality.

MDR proteins are associated with cell longevity.
With ageing, MDR proteins are less functional, causing accumulation of intracellular toxins within the cell.

MDR mode of action

MDR are specialized cellular pumps of the cell membrane that find toxins and eject them outside, for safe disposal.
It sits searches for foreign hydrophobic molecules.

When it finds one, it grabs the molecule in a pocket deep within the protein, and then flips to a new conformation. The new conformation has an opening towards the outside of the cell, and the molecule is ejected. The whole process is powered by ATP to ensure that everything happens in a timely manner.



PDB doi: 0.2210/rcsb-pdb/mom-2010-3

MATERIEL & METHODS

Given the different MDR1 properties, we assumed that increasing MDR1 activity could be a discerning anti-ageing strategy. To assess this assumption, we screened compounds for their ability to stimulate MDR1 activity on primary fibroblast cells. The compounds allowing the higher stimulation of MDR activity were then mixed to treat cells under the same protocol before the genomic assay was performed, looking for modulations of genomic expression.

Cell culture

Primary fibroblast cells from tebu-bio (Cat 106-05a – from facial plastic surgery on a 42 years old Caucasian woman) are cultured using the Fibroblast Growth Medium (tebu-bio - Cat. 95116500) according to the supplier's recommendations.

Genomic Assay

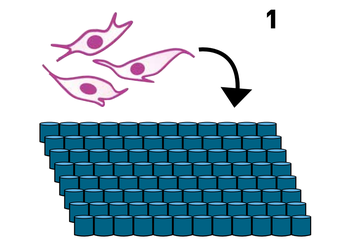
D0: Primary fibroblast cells passage 4 are split in 2 T75 flasks ($3{,}5.10^6$ cells/flask) and incubated overnight (37°C, 5% CO_2) for cell adhesion.

D1: medium is replaced with same fresh medium in the 1st flask. In the second flask D-Glyox complex was added to the culture medium.
D2 : cells are trypsinized, PBS rinsed, centrifuged, and replaced in PBS before liquid N2 cryconservation.

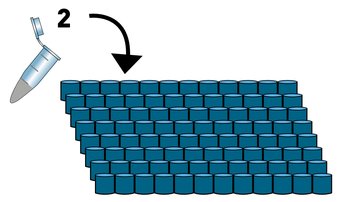
Tebu-bio performed the genomic assay to evaluate ARN modulation with the microarray RT² Profiler™ PCR Array Human Cellular Senescence (PAHS-050Z).

MDR Assay

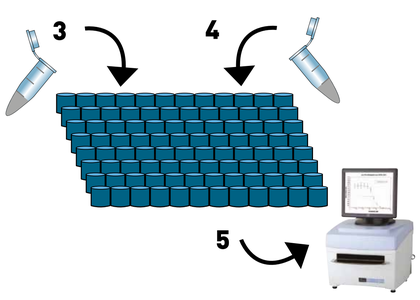
MDR activity is evaluated using the Molecular Probes Vybrant MDR Resistance Assay Kit (V13180 Invitrogen Life Science) following next protocol :



D0 : Cell Seeding (1)
7000 cells passage 4 are seeded in culture-treated clear-bottom black 96-well plates (Corning) and incubated 48h at 37°C, 5% CO2 for adhesion.

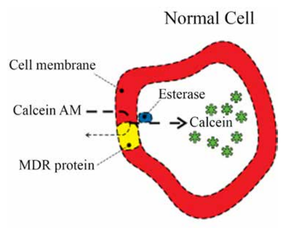


D2 : Cell Treatment (2)
Culture medium is replaced by 150 µl/well of active agent diluted in culture medium.
Verapamil (30 µg/ml) is used as negative control.
FGF (Sigma - 5 nM) and KGF (Sigma - 25 ng/ml) are used as positive controls.

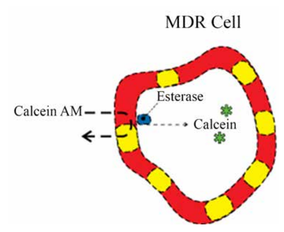


D3 : Calcein MDR activity Assay
50 µl of calcein diluted to 1 µM are added in each well (3).
After one hour incubation, cells are rinsed twice before introducing 150 µL lysis buffer solution (water 0.25M of sucrose (Sigma), and 0.1M EDTA (Sigma)) (4).
Calcein retention is measured on a Perkin Elmer Enspire® Multimode Reader fluorescence spectrophotometer (λex: 494 nm, λem: 512 nm) (5).

Principle of Molecular Probes Vybrant MDR Resistance Assay (V13180 Invitrogen Life Science).



In normal cells, non-fluorescent calcein enters the cell and accumulates therein.
It is hydrolyzed inside by endogenous esterases, and becomes fluorescent.



In cells with increased MDR expression, expulsion of calcein out of the cell before it is cleaved is made possible: there is no fluorescence left

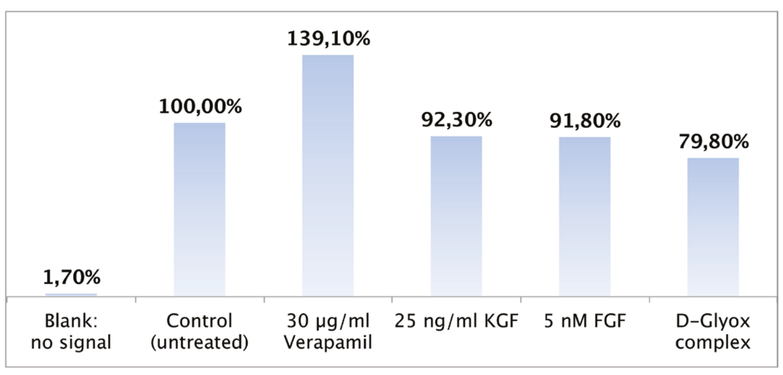
MDR activity is calculated as follow:
f_0 being the basal calcein level, measured in the wells containing untreated cells
fAx being the ratio measured for each of the other wells (cells treated with compounds).
% calcein retention = fAx x 100 /f_0

➥The weaker is the fluorescence, the more active are the MDRs.

RESULTS & DISCUSSION

MDR Activity Assay

We have screened different compounds on the MDR activity assay to finally design D-GlyOx. It is composed of two vegetal extracts combined with a peptide. D-GlyOx complex has been used to treat cells for the genomic assay.



The D-GlyOx complex has a higher capacity to enhance MDR activity than KGF and FGF positive controls (+20.2% versus 8,2% for FGF 5nM; negative control with Verapamil 30µg/mL reducing MDR activity of 39%).

Genomic Assay

After treatment with the D-GlyOx complex, 5 RNAs are significantly modulated (see the table below).
Inhibition of IGFBP3 RNA absolutely reflects a protection from senescence. The RNA over expression of FN1, THBS1, and COL1 reflects an improvement for dermal adherent and structure protein synthesis. It is the proof that cell metabolic functions are stimulated. The increased expression of CDKN2B RNA is more subject to discussion. Considering former RNA modulations, one can consider that cell growth is inhibited in order to promote protein synthesis.

Gene	Pathway	Detail	Fold increase	Effect
IGFBP3	Senescence initiators: IGF related	Senescence initiators: p53/PRB signaling (cell cycle)	-2,41	☺
FN1	Senescence responses : Cytoskelton related	Fibronectin binds cell surfaces and various compounds (collagen, fibrin, heparin...). It is involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. THBS1 encodes for the thrombospondin 1 adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions, bounding sefveral coumpounds (fibrinogen, fibronectin, laminin, type V collagen...). Collagen has not to be presented.	2,22	☺
THBS1	Senescence responses : Cytoskelton related & Cell adhesio		2,13	☺
COL1	Senescence responses : Cell adhesion		2,06	☺
CDKN2B	Senescence initiators: p53/PRB signaling (cell cycle)	Encodes the cyclin-dependent kinase inhibitor 2B, which prevents the activation of the CDK kinases. The encoded protein functions as a cell growth regulator that controls cell cycle G1 progression.	2,11	😐

CONCLUSION

Globally, the D-GlyOx complex, enhancing MDR activity, confirms a positive effect on fibroblast cells: the genomic evaluation correlates with a reduction in senescence markers.
We notice a growth inhibition, and we observe a clear improvement in fibroblast capacity to produce dermal adherent and structure proteins.

Finish products formulated with the D-Glyox complex

Altogether, our results confirm that the strategy of MDR activity stimulation to maintain an active synthesis of cell metabolite and to delay senescence is relevant.

Therefore, enhancing MDR activity is a good strategy to improve cell detoxification system, ensuring cell regeneration and thus protecting cellular longevity.

It would be of interest to achieve this study working on a cell senescent model. We would then examine if an enhancement of MDR activity leads to senescence recovery.

Acknowledgment

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tebu-bio
Innovative Lab Services & Reagents

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