Molecular pathways of response and potential mechanisms of resistance to the HSP90 inhibitor, Celastrol, in Hodgkin and Reed-Sternberg cells

Priscilla Segges[a], Stephany Côrrea[b], Bárbara Du Rocher[b,c], Gabriela Vera-Lozada[a], Flavia Krsticevic[a,d], Debora Arce[d], Eliana Abdelhay[b], Rocio Hassan[a]

aOncovirology Laboratory, CEMO, INCA , Rio de Janeiro, Brazil; bLaboratório Célula-Tronco, CEMO, INCA, Rio de Janeiro, Brazil; cLaboratório de Pesquisa sobre o Timo, FIOCRUZ, Rio de Janeiro, Brazil; d CIFASIS - Centro Internacional Franco Argentino de Ciencias de la Información y de Sistemas, Rosario, Argentina

INTRODUCTION

Classical Hodgkin lymphoma (cHL) is an unusual malignancy in that the tumor cells, the Hodgkin and Reed-Sternberg cells (H-RS), account for a minor component of the tumor mass, the bulk of which is a mixed cellular infiltrate[1]. Most patients with cHL (80%) enjoy durable remissions following front-line treatment, however, relapsed or refractory disease is a challenging problem with a poor prognosis and limited therapeutic options[2]. In addition, the development of less toxic therapeutics agents is an ongoing goal because current therapy is associated with toxicity and secondary malignancies.

In cancer chemoprevention, the use of natural compounds represents a promising strategy in the search for novel therapeutic agents[3]. Celastrol, a triterpene derived from the Chinese medicinal plant *Triterygium wilfordii*, has been identified as a novel inhibitor of Heat-shock protein 90 (HSP90) and has attracted great attention lately for its potent anti-tumor effects[4]. Here, the effects of celastrol, were determined on cHL-derived cell lines (KM-H2 and L428). We also applied a proteomic approach to reveal the potentials targets being modulated by celastrol.

METHODS

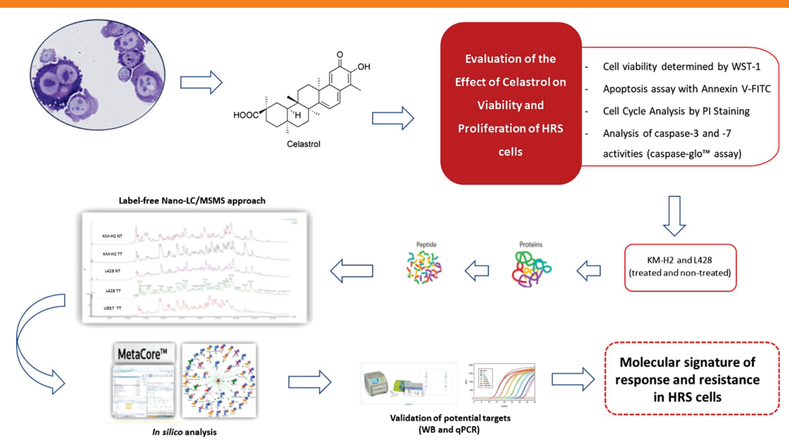

Figure 1. *Study* design.

RESULTS

Effects of celastrol on KM-H2 and L428 cells

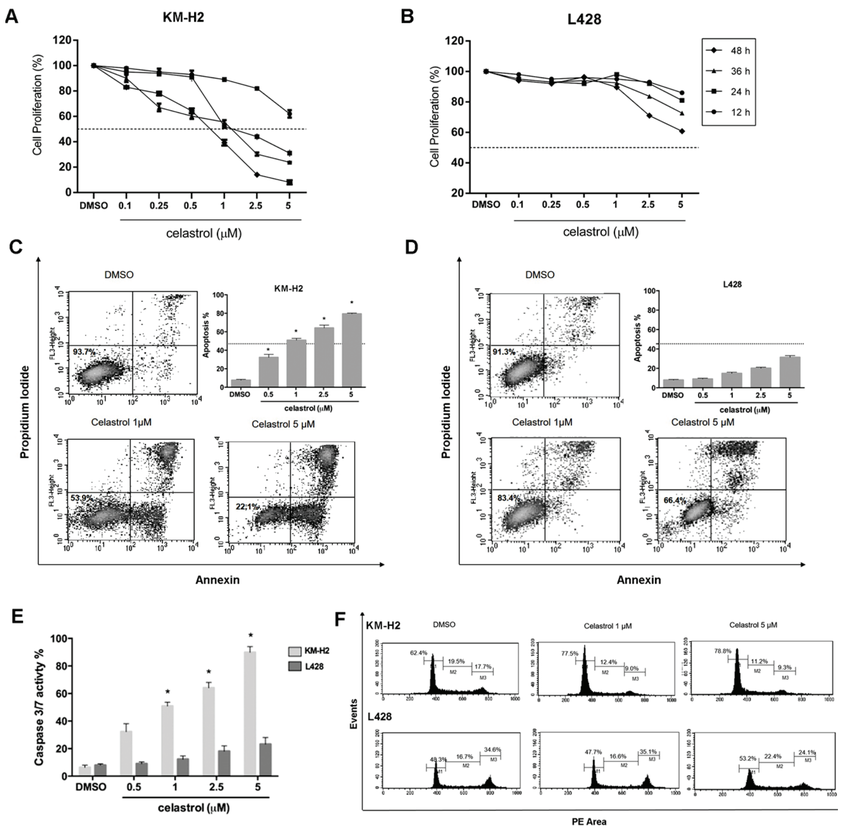

Figure 2. Effects of celastrol on KM-H2 and L428 cells. KM-H2 (A) and L428 (B) cell lines were treated with the indicated concentrations of celastrol or the vehicle control (DMSO) for 24, 48 and 72 h, and cell viability was detected by WST-1 assay. Apoptosis of KM-H2 (C) and L428 (D) cell lines induced by celastrol (0.5, 1, 2.5 and 5μM) determined by the Annexin V assay after 24h. Cell lines incubated with vehicle control (DMSO) were used as control of spontaneous apoptosis. The images are representative of three independent experiments and the means and errors of all the independent experiments are shown in the column graphical. Percentage of celastrol-induced cell death was calculated by subtracting the spontaneous death in the control from the overall cell death in the celastrol-treated samples for each dose point. E) Profile of caspase-3/7 activation mediated by celastrol compound in KM-H2 and L428 cells. The percentages of celastrol caspase-3,7 activation was calculated by subtracting the values in the caspase positive from the negative control sample (DMSO). F) Analysis of cell cycle profile changes induced by the celastrol compound in KM-H2 and L428 cells. The cell lines were exposed to indicated concentrations of celastrol and to DMSO and collected after a 24-h exposure. One representative of 3 independent experiments is shown. The values shown are the mean of three independent experiments. Error bars represent ± standard error (*p<0.01, **p<0.001).

Celastrol induces changes in the proteome of Hodgkin's lymphoma cell lines

- We shown that celastrol perturbs multiple signaling pathways, which mainly involves the MAPK kinase pathway, metabolism, dysregulation of protein folding, proteolysis, protein trafficking and cytoskeleton organization.
- However, the major effect was to modulate the protein homeostasis and the stress response pathways.

Table 1. Differentially expressed proteins in KM-H2 and L428 cell lines compared to the treated and untreated condition.

differentially expressed proteins	KM-H2	L428
up-regulated	121	2
down-regulated	6	182
unique in TT	87	79
unique in NT	48	81
total	262	344

Table 2. Representative Biological process modulated in KM-H2 and L428 cell lines.

#	Biogical processes	FDR	N# proteins in total	N# proteins identified
KM-H2 cell line				
1	posttranscriptional regulation of gene expression	5,542E-13	669	39
2	chromosome organization	9,420E-12	1344	53
3	organelle organizati on	9,420E-12	4189	103
4	regulation of organelle organization	1,119E-11	1713	60
5	regulation of cellular component organization	1,189E-11	3298	88
6	cellular protein metabolic process	1,189E-11	4577	108
7	Fc receptor signaling pathway	1,316E-11	361	27
8	cellular component organization or biogenesis	1,316E-11	7183	144
9	cell cycle	7,472E-11	1770	59
10	cellular metabolic process	7,518E-11	10816	186
L428 cell line				
1	translational initiation	1,170E-24	173	32
2	SRP-dependent cotranslational protein targeting to membrane	1,079E-20	103	24
3	protein localization to endoplasmic reticulum	1,080E-20	148	27
4	viral gene expression	1,553E-20	136	26
5	nuclear -transcribed mRNA catabolic process, nonsense -mediated decay	1,553E-20	137	26
6	viral transcription	1,691E-20	124	25
7	cotranslational protein targeting to membrane	1,691E-20	110	24
8	protein targeting to ER	7,170E-20	117	24
9	establishment of protein localization to endoplasmic reticulum	1,841E-19	122	24
10	cellular component biogenesis	9,278E-19	3351	106

*Process listed in the table are those statistically most relevant using the Metacore Analysis. FDR: False discovery rate.

Table 3. Representative Pathways modulated in KM-H2 and L428 cell lines.

Cellular Function	Pathway Name*	FDR	N# proteins data/ total	Identified Proteins
KM-H2 cell line				
Development Biology	Development_Ligand-independent activation of ESR1 and ESR2	2,514E-05	8/44	p300, ERK1/2, ERK1 (MAPK3), ERK2 (MAPK1), PI3KIA, PI3KIA t class IA (p110-alpha), p90RSK1, CBP
Immune response	NETosis in SLE	3,989E-04	6/31	ERK1/2, Histone H3, Histone H2, Histone H2A, Histone H1.2, Histone H1
Cell cycle	Cell cycle_Role of Nek in cell cycle regulation	3,989E-04	6/32	Histone H3, PI3K cat class IA, Tubulin, Tubulin beta, Histone H1, Tubulin alpha
Development Biology	Cytoskeleton remodeling_Neurofilaments	1,687E-03	5/25	Vimentin, Tubulin (in microtubules), Tubulin beta, Desmuslin, Tubulin alpha
Signal Transduction	Signal transduction_Additional pathways of NF-kB activation	2,915E-03	5/30	p300, ERK1/2, Histone H3, p90RSK1, CBP
Development Biology	Development_IGF-1 signaling	2,915E-03	6/50	ERK1/2, ERK1 (MAPK3), ERK2 (MAPK1), PI3K cat class IA, NF-kB, CDC42
Immune response	Sorafenib-induced inhibition of cell proliferation and angiogenesis in HCC	2,915E-03	4/16	VEGFR-1, ERK1/2, ERK1 (MAPK3), ERK2 (MAPK1)
Cell cycle	Cell cycle_Start of DNA replication in early S phase	2,915E-03	5/32	RPA3, MCM3, Histone H1, MCM5, MCM2
Signal Transduction	Signal transduction_Activin A signaling regulation	2,915E-03	5/33	p300, Histone H3, Evi-1, Histone H2, CBP
Development Biology	Development_S1P1 receptor signaling via beta-arrestin	2,915E-03	5/34	ERK1/2, ERK1 (MAPK3), ERK2 (MAPK1), PI3K cat class IA (p110-alpha), p90Rsk
L428 cell line				
Metabolism of proteins	Regulation of degradation of deltaF508-CFTR in CF	3,525E-05	8/39	HSP70, HSP105, HSP27, SUMO-2, E2l, Aha1, SAE1, BAG-2
Immune response	NETosis in SLE	4,300E-04	7/31	ERK1/2, Histone H3, Histone H2A, Histone H2, Histone H1, Histone H1.2, HMGB1
Transcription	Transcription_Negative regulation of HIF1A function	4,906E-04	8/66	HSP70, MCM7, PSMA7, PRDX4, RUVBL2, MCM2, MCM5, PRDX2
Cell cycle	Cell cycle_Start of DNA replication in early S phase	1,211E-03	6/32	MCM4/6/7 complex, RPA3, MCM2, MCM4, Histone H1, MCM5
Development Biology	Development_Regulation of cytoskeleton proteins in oligodendrocyte differentiation and myelination	1,673E-03	7/58	Tubulin alpha, Tubulin, Actin cytoskeletal, Tubulin beta, Dcc, MRLC, Cortactin
Cytoskeleton remodeling	Cytoskeleton remodeling_Neurofilaments	2,510E-03	5/25	Tubulin alpha, Tubulin, Actin cytoskeletal, Tubulin beta, Kinesin heavy chain
Immune response	Immune response_Sublytic effects of membrane attack complex	3,169E-03	7/68	RK1/2, GRP75, HSP27, Actin cytoskeletal, cPLA2, GRP78, eIF2S1
Development Biology	Development_Slit-Robo signaling	3,169E-03	5/30	Tubulin, Actin cytoskeletal, Actin, ACTB, Cortactin
Transport	Transport_The role of AVP in regulation of Aquaporin 2 and renal water reabsorption	3,543E-03	6/50	ERK1/2, Actin cytoskeletal, ACTB, MRLC2, MRLC, Annexin II
Cell cycle	Cell cycle_Role of Nek in cell cycle regulation	3,525E-05	5/32	Tubulin alpha, Tubulin, Histone H3, Tubulin beta, Histone H1

*Pathways listed in the table are those statistically most relevant using the Metacore Analysis. FDR: False discovery rate.

Validation of proteomic results

- Celastrol's toxicity was associated with down-regulated expression of RAS, ERK1/2 and c-Fos, which we identify as the mechanism of celastrol-mediated apoptosis in KM-H2.
- A correlation was observed between HSP27 expression and the response or resistance to celastrol in cHL cell lines, where L428 cells displayed a strong increase in HSP27 levels, whereas a prominent decrease was seen in KM-H2 cells, at protein and mRNA levels

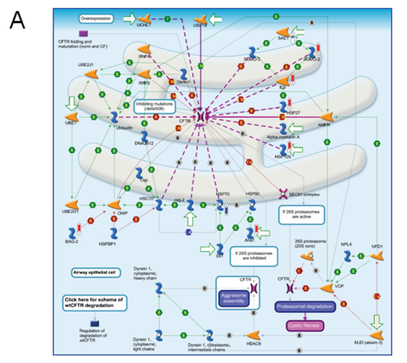

Figure 3. Representative Pathways modulated in KM-H2 (B) and L428 (A) cell lines.

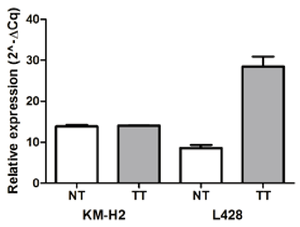

Figure 5. mRNA levels were analyzed by real-time PCR 24 hours after treatment with celastrol. NT: untreated and TT: treated. Bars: SD. * differ from control P < 0.001.

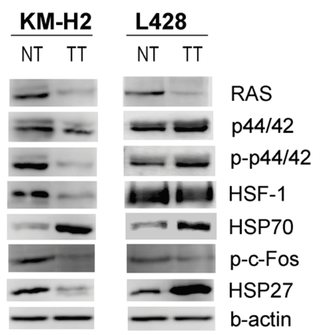

Figure 4. Western blot analysis of differentially expressed proteins found in our proteomic study. KM-H2 and L428 cells were treated with 1μM of celastrol for 24h for validation of potential markers based on quantitative MS-data. Samples (30μg) were separated by SDS-PAGE and probed with specific antibodies, as indicated. NT: non-treatment. TT: treatment.

CONCLUSIONS

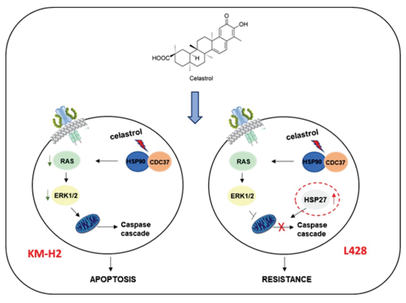

Figure 7. Proposed model for the effects celastrol on HRS cells.

- This study provides the first evidence of the potential role of celastrol, a HSP90 inhibitor, in regulating the growth and survival of H-RS cells.
- Our results indicate that celastrol can promote the apoptosis in KM-H2 cells by down-modulating the MAPK/ERK pathway and that resistance may emerge in part due to compensatory mechanism involving activation of HSP27.
- Our work suggests celastrol as a promising anti-tumoral compound and disclose HSP90 and HSP27 inhibitions as candidate targets in cHL.

REFERENCES

1. R. Kuppers, Nature reviews. Cancer 9 (2009) 15-27.
2. I. Schwering, et. al. Blood 101 (2003) 1505-1512.
3. M. Fridlender, et. al. Frontiers in plant science 6 (2015) 799.
4. T. Zhang, A. et. al. Molecular cancer therapeutics 7 (2008) 162-170.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA