Research Article

International Journal of Research in Oncology

Cytotoxicity Assessment of Sea Star Asterias Rubens Protein-Sip-Young-6 HIS in Hela Cells. Comparisons with Doxorubicine Action

Michel Leclerc*

Immunology of Invertebrates, Orléans University, France.

***Correspondence:**
Michel Leclerc, Immunology of Invertebrates, Orléans University, France.

Received: 18 May 2022; **Accepted:** 22 Jun 2022; **Published:** 26 Jun 2022

Citation: Leclerc M. Cytotoxicity Assessment of Sea Star Asterias Rubens Protein-Sip-Young-6 HIS in Hela Cells. Comparisons with Doxorubicine Action. Int J Res Oncol. 2022; 1(1): 1-3.

ABSTRACT

The objective of this work is to assess whether recombinant protein SIP-Young-6 HIS, produced in HEK293, induces cell death in HeLa cells, when compared to doxorubicine action to achieve this, the metabolic activity is measured with the Cell Cytotoxicity Assay Kit (Abnova, Ref# KA4151) which contains a water-soluble dye that changes its absorption spectra upon cellular reduction.

It seems that a post-translational modification occurs in HEK 293 since obtained results with the sea star young protein were less convincing than the preliminary ones. These last used directly the sea star IGKappa gene cloned in a CMV vector.

Keywords

Invertebrate sea star, Vertebrates, HELA cells, Cloning, Doxorubicine.

Introduction

In a very recent work, we have tested the effects of sea star recombinant protein SIP-Young-6 His produced in HEK 293 cells [1] against MCF7 cells [2].

In the present work, we try to prove the sea star recombinant protein has a spontaneous cytotoxicity against Hela cells as it was shown by the use of young sea star protein cloned in CMV vector.

Materials and Methods

SIP-Young-6-HIS protein produced in HEK293 and provided at 0.13mg/ml in PBS buffer was used.

A Cell Cytotoxicity Assay Kit from Abnova, Ref# KA4151 and lot number 187115 was performed against Hela cells.

We Recall That

HeLa cell line was grown in T75 flasks with MEM alpha media, with 10% Fetal Bovine Serum (FBS) and gentamicin. Detachment was performed with trypsin, after extensive washing with DPBS. Once cells were detached, trypsin was inactivated with the FBS from the cell culture media. HeLa is an epithelial cell line derived from cervical cancer cells.

Doxorubicin (Dox) Was Equally Used

Doxorubicin (DOX) [2] is involved in the induction of DNA damage, inhibition of cell proliferation, impairment of mitochondria, and cell death, and is therefore used as a cell-death inducer. This last was compared to our young sea star recombinant in the cytotoxic assay, which is described now.

Cells from T75 flasks were seeded on 96-well plates with black walls and clear bottom. Cells were seeded at a density of $1{,}5 \cdot 10^4$ cells in 100uL cell culture media.

Both DOX and the recombinant protein were tested in different concentrations, using DPBS as diluent. Concentrations were adjusted so all wells were treated with 10uL of DOX, protein solution, or DPBS (vehicle C-) for 24h. All conditions were tested in triplicates following the scheme.

As DOX may interfere with the spectrophotometry reading, after the 24h treatment all wells were carefully washed with DPBS twice.

Concentrations in mg/mL

[DOX]		1	2	3	4	5	6	7	8	9	10	11	12
[SIP-Young-6His]	A	1			0,0078			0,13			0,00102		
	B	0,500			0,0039			0,065			0,00051		
	C	0,250			0,00195			0,0325			0,00025		
	D	0,125			0,00098			0,01625			0,00013		
	E	0,063			0,00049			0,00813			0,00006		
	F	0,031			0,00024			0,00406			0,00003		
	G	0,016			0,00012			0,00203			0,00002		
	H	Vehicle control						No cells control					

570/605 ratio		1	2	3	4	5	6	7	8	9	10	11	12
	A	1.008	0.975	0.893	0.928	0.921	0.937	3.129	3.312	2.838	2.697	2.351	2.139
	B	0.834	0.835	0.835	1.140	1.209	1.154	3.119	2.961	2.943	3.086	2.739	2.387
	C	0.796	0.795	0.793	1.479	1.401	1.400	3.063	3.032	2.955	2.935	2.661	2.190
	D	0.795	0.793	0.792	1.926	1.822	1.923	2.755	3.097	2.845	2.713	2.196	1.978
	E	0.795	0.795	0.792	2.206	2.063	2.185	2.758	2.725	2.655	2.552	2.498	1.936
	F	0.797	0.794	0.797	2.486	1.965	2.592	2.632	2.579	2.642	2.547	2.305	1.993
	G	0.819	0.862	0.838	2.675	2.618	2.682	2.627	2.684	2.654	2.582	2.680	2.272
	H	2.493	2.504	2.594	2.445	2.449	2.369	0.795	0.790	0.788	0.789	0.788	0.787

DOX dose (µg/well)	% Cell viab	Protein dose (µg/well)	% Cell viab
100	9.6	1.3	136.2
50	2.7	0.65	131.6
25	0.3	0.325	132.2
12.5	0.2	0.1625	124.9
6.25	0.3	0.08125	114.1
3.125	0.4	0.040625	108.5
1.5625	2.8	0.020313	110.7
0.78125	8.3	0.010156	94.4
0.39063	22.4	0.005078	114.4
0.19531	37.8	0.002539	105.6
0.09766	65.3	0.00127	87.6
0.04883	80.8	0.000635	89.7
0.02441	90.9	0.000317	87.5
0.01221	110.9	0.000159	101.7
0 (Vehicle control)	100.0	0 (Vehicle control)	100.0

One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	39.49				
R squared	0.6810				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	37.54				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	14.51	2	7.256		
Residual (within columns)	6.798	37	0.1837		
Total	21.31	39			
Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
PBS vs DOX	1.166	6.913	Yes	***	0.7776 to 1.554
PBS vs SIP-Young-6His	-0.1660	0.9843	No	ns	-0.5540 to 0.2221

After that, 100 µL cell culture media and 20 µL Cell Cytotoxicity Assay Kit are added. The spectrophotometry reading is performed after 1, 2 and 3h of incubation, as the Cell Cytotoxicity Assay Kit incubation time is dependent upon the cell type.

Results

They are quite similar among the 3 readings, so the 2 hours timepoint is used for the result analysis.

The vehicle control is stablished as the reference for the rest of the conditions and is therefore assigned a 100% viability. Cell viability for the rest of experimental conditions is calculated as:

$$(Sample_{Mean\ Abs} - No\ cells_{Mean\ Abs}) / (Vehicle_{Mean\ Abs} - No\ cells_{Mean\ Abs}) \times 100$$

This data is analyzed by a One-way ANOVA and the Dunnett's Multiple Comparison Test, used to compare different groups against a control group (in this case, the negative control). Although the means and variances are significantly different among groups, the Dunnett's test is significant for DOX but not for SIP-Young-6His.

Conclusion

A decreased cell viability is observed with higher DOXO amounts, indicating that the assay works properly.

When the DOX dose is too high (100 and 50µg/well) the viability seems to increase again. It is probably due to an artifact: the cells were visibly DOX-stained by the drug even after the washings, and this may affect the spectrophotometry reading. On the other hand, the 24h treatment with SIP-Young-6-His protein at the stated doses is slightly increasing HeLa viability. However, this increase is not significantly different to the DPBS effect, according to the Dunnett's post-hoc test. Nevertheless, we recall that the young protein issued and cloned in CMV vector had a high and significant cytotoxicity against Hela cells. We conclude our young recombinant sea star protein has been modified by cloning in HeK293, and, its main properties we know today have been lost, due to a post-translational modification in HeK cells.

References

1. Leclerc M. Recombinant Sip Young 6 His Protein Production from the Sea Star Igkappa Gene, after Cloning. Eur J Biol and Biotechnol. 2021; 2: 85-86.
2. Carrie J Lovitt, Todd B Shelper, Vicky M Avery. Doxorubicin resistance in breast cancer cells is mediated by extracellular matrix proteins. BMC Cancer. 2018; 18: 41.