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Short Communication

Cytotoxicity Assessment of Sea-Star Protein SIP-Young-6His in MCF-7 Cells

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ABSTRACT

The objective of this project is to assess whether recombinant protein SIP-Young-6His, produced in HEK293, induces cell death in MCF-7 cells. 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. In first analysis it seems that sea star protein SIP-Young-6His have a weak cytotoxicity against MCF7 cells when compared to Doxorubicin activity against these same tumoral cells.

Keywords: Sea star; MCF7 vertebrate cells; Doxorubicin; Invertebrates

INTRODUCTION

Cytotoxic activity of sea star axial organ, of sea star IGKappa gene have been demonstrated against Mouse tumoral cells and human ones [1,2]. In the present work, we had tried to study the cytotoxicity of sea star: SIP-Young-6His protein produced in HEK293 and provided at 0.13 mg/ml in PBS buffer [3] against MCF-7 cells when compared to doxorubicin [4].

MATERIALS AND METHODS

A Cell Cytotoxicity Assay Kit from Abnova, Ref# KA4151 and lot number 187115 was used. Second, MCF-7 cell line was grown in T75 flasks with DMEM: F12 (1:1) 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. MCF-7 is an epithelial cell line isolated from the breast tissue of a patient with metastatic adenocarcinoma. These cells were taken as targets. Doxorubicin (DOX) (Clinisciences) involved in the induction of DNA damage, inhibition of cell proliferation, impairment of mitochondria, and cell death, and was therefore used as a cell-death inducer [3] so, 2 cell-death inducers were compared: Doxorubicin and sea star SIP-Young-6His protein.

RESULTS

Cytotoxicity assay and results

Cells from T75 flasks were seeded on 96-well plates with black walls and clear bottom in 100 uL cell culture media to reach a confluency of 70% after 48 h of culture. A first cytotoxicity test was performed to assess which is the DOX amount necessary to reach a metabolic activity decrease, or cell death, of around 50% (Tables 1-6).

DOX Treatment (ug)	1	2	3	4	5	6
A	10	5	2,5	1,25	0,625	0,1325
B						
C						
D	Vehicle C-					

Table 1: DOX was dissolved in DPBS and concentrations were adjusted so all wells were treated with 10 uL of DOX solution or DPBS (vehicle C-), in at least triplicates.

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Ratio Abs 595/620 nm	1	2	3	4	5	6
A	3,503	3,924	4,367	4,721	4,632	3,820
B	3,470	4,140	4,538	4,657	4,811	3,927
C	3,536	4,099	4,746	4,734	4,952	3,740
D	4,136	4,645	4,819	4,816	4,783	**2,671**

Table 2: The Cell Cytotoxicity Assay Kit is added, and the spectrophotometry reading is performed after 24 h of DOX and Cell Cytotoxicity Assay Kit incubation following manufacturer's instructions. *The bold value is not used for the cell viability calculations since it is out of the tendency.

These results are used for the cell viability calculation, which is calculated as: Sample Mean Abs/Vehicle Mean Abs × 100

DOX dose (µg/well)	% Cell viability
10	76
5	88
2,5	98
1,25	102
0,625	104
0,3125	83
0 (Vehicle C-)	100

Table 3: It is showing that higher DOX doses are related to lower cell viabilities.

The cell death effect from DOX is no longer observed for doses of 1.25 µg/well or lower, except for dose 0.31 µg/well, when lower cell viability is observed again.

DOX Treatment (ug)	1	2	3	4	5	6
A	10	50	25	12,5	6,25	3,125
B						
C						
D	Vehicle control					
E	1,3	0,65	0,325	0,1625	0,0813	0,041
F						
G						
H	No cell control					

Table 4: Increased DOX concentrations are tested for the final assay to reach lower cell viabilities. Also, the SIP-Young-6His protein (in PBS) is tested in different dilutions and some wells with no cells are added as background controls.

As DOX may interfere with the spectrophotometry reading, after the 24 h treatment all wells are carefully washed with DPBS twice. After that, 100 µL cell culture media and 20 µL Cell Cytotoxicity Assay Kit are added.

Ratio Abs 595/620 nm	1	2	3	4	5	6
A	1,167	0,897	0,901	0,951	1,503	1,855
B	1,049	0,886	0,939	0,986	1,504	1,987
C	1,060	0,944	0,847	0,939	1,385	1,987
D	1,897	1,864	**2,187**	1,898	1,841	**2,150**
E	2,039	2,153	1,937	1,733	1,876	1,808
F	2,069	2,088	1,909	1,887	1,792	1,780
G	1,964	1,988	1,838	1,737	1,785	1,818
H	0,8145	0,8359	0,807	0,8069		

Table 5: The spectrophotometry reading is performed after 1, 2 and 3 h of incubation, as the Cell Cytotoxicity Assay Kit incubation time is dependent upon the cell type. No relevant differences among wells are observed after 1 and 2 hours of incubation with the reagent, so the 3 hours' time point is used for the result analysis. *The bold values are out of the tendency for the vehicle control and are therefore dismissed.

Cell viability calculation is calculated as: (Sample Mean Abs-No cells Mean Abs)/(Vehicle Mean Abs-No cells Mean Abs) × 100.

Dose (µg)	% Cell viab	Protein dose (µg)	% Cell viab
0	100		
100	26	1,3	114
50	9	0,65	119
25	7	0,325	102
12,5	13	0,1625	91
6,25	61	0,0813	95
3,125	106	0,0406	93

Table 6: Cell viability of normal dose and protein dose is calculated here.

DISCUSSION AND CONCLUSION

Decreased cell viability is observed with higher DOXO amounts, indicating that the assay works properly. On the other hand, the treatment with sea star SIP-Young-6-His protein, after 24 h with the stated doses, does not seem to be affecting the cell viability of MCF-7 cells. We observe a weak cytotoxicity against these last cells. Further studies are necessary to complete the study of Spontaneous and Induced cytotoxicity of the invertebrate protein issued from Hek cells against cancerous vertebrate cells.

We notice that: "Spontaneously occurring cytotoxicity of sea star axial organ cells on target cells has been studied. It appears that in an allogeneic system no cytotoxic phenomenon occurs after 4 or 6 h of culture, whereas spontaneous cytotoxicity toward

malignant vertebrate target cells is shown and reaches its maximum at 37 degrees C. In a certain way, the axial organ cell population includes cells, which may be compared with vertebrate natural killer cells. On the other hand, it must also be noted that axial organ cells are able to exert in a few cases, an induced cytotoxicity on MBL2 cells. This phenomenon appears at the end of the fourth day of culture".

REFERENCES

1. Leclerc M, Luquet G. Effect of in vivo inoculation of bacteria on the spontaneous cytotoxicity of axial organ cells from Asterias rubens. Immunol Lett
2. .1983;6(2):107-8.
3. Leclerc M. Cell-mediated and humoral immune responses in the sea-star Asterias Rubens (Echinoderm): notion of invertebrate primitive antibody. SAJ Biotechnology. 2015;2(1):1.
4. Leclerc M. Recombinant Sip young 6 His protein production from the sea star igkappa gene, after cloning. Eur J Biol.2021;2(2):85-6.
5. Lovitt CJ, Shelper TB, Avery VM. Doxorubicin resistance in breast cancer cells is mediated by extracellular matrix proteins. BMC cancer. 2018;18(1):1-1.