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Mini-Review

Heterotransplantations and Genomic. The Asterias Rubens Sea Star Axial Organ (a.o) Cells, the Sea star Igkappa Gene: Effects against Malignant Vertebrate Cells. Preliminary Review.

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Abstract

We performed in 1975, the first heterotransplantation of invertebrate A.O in nude mouse, then a double heterotransplantation of human tumor and axial organ next to this last one, always in nude mouse: The human tumor was rejected in 50% of observed cases. Some years later, we found that A.O cells exerted an induced and spontaneous cytotoxicity against SP2 and MBL2 mouse tumoral cells. Recently, we discovered a sea star Igkappa gene with immune properties. This gene was inserted in a CMV (cyto-megalovirus) and finally in a plasmid called « young » plasmid.

The induced « young » protein exerted a spontaneous cytotoxicity against osteosarcom cells (U2oS cells) against A-375 mel-anome cells and Hela cells.

Introduction

In 1975, Leclerc transplanted with success an invertebrate organ: the sea star axial organ(a primitive lymphoïd organ) in nude mouse [1] then risked a double heterotransplantation always in nude mouse: it was composed of a sea star axial organone and a human tumor skin one besides the precedent one, under the skin of nude mouse, in back position. (Unpublished results) In 1983, Luquet and Leclerc [2] shown that the axial organ cells (AO cells), exerted a spontaneous and induced cytotoxicity against mouse SP2 myeloma cells and MBL2 cells.

The AO cells included essentially lymphocytes and phagocytes [1] 40 years later, we discovered a sea star Igkappa gene [3], with immune properties [4] We have studied the behavior of the « young » protein secreted by the sea star Igkappa gene, in front of human malignant: A-375 melanome cells, human Osteosarcome cells (U2oS cells) and human malignant cells Hela, by the use of plasmids.

Materials and Method

Animals

a) Asterias rubens, a sea star was collected at Arcachon (France)/ the A.O (axial organ) was excised With pinces and passed or not in antibiotics (peni-streptomycin) according to the used method.
b) Nude mice were purchased by the CSEAL-CNRS Orléans.-La source.
c) Mouse SP2 and MBL2 cells were cultured in our laboratory.
d) Gene cloning in a cytomegalovirus (CMV) was done as seen in (fig. 1 and 2), from the sea star Igkappa gene [3]. It constitutes the « promoter ».

Following steps as plasmid realization in correlation with the promoter, plasmid amplifications, transfections [5] were performed.

A-375 human melanome cells were used. They were transfected by plasmids, after electroporation, at time t=0. (AMAXA process)

[6] Or by classical electroporation for U2oS cells and Hela cells. At time t=24 h, Cell suspensions were put on slides. Observations were realized with an optical microscope or by spectrophoto metry.

Cloning in N-terminal pCMV-Tag3B (c-mic tag)/ BamH1-EcoR1

GGA TCC GGA GGA ATG CGTGGCAACATGGCGTCTCTATGGATGTTCTTCTTTGTCGTGGGGATAACTTTACAACGGAGT TTGGCGATTTACAC-GTTTCGCGAGCAACCGTCGGACACTAGCGCGTTGCAGGGGAGCACAGTGGTGCTTCACTGCTCCGTTGAGCAGTACATAAACACCACGGCCATC-GTTTGGTGGAGCCGTGACTCGGTCATCGCCACAACAAAGACCTGAAACTGTCCAGTCT AAACACCGACCAGCTCCAAAGGTACTCGATTTCAGGC-GACGCATCTCGGGGGGAATTCAACCTTAAAATAGTGAACTTTACCG CCACAGACGCCGCCAGTTACCGCTGTCAGATG TAA GAA TTC

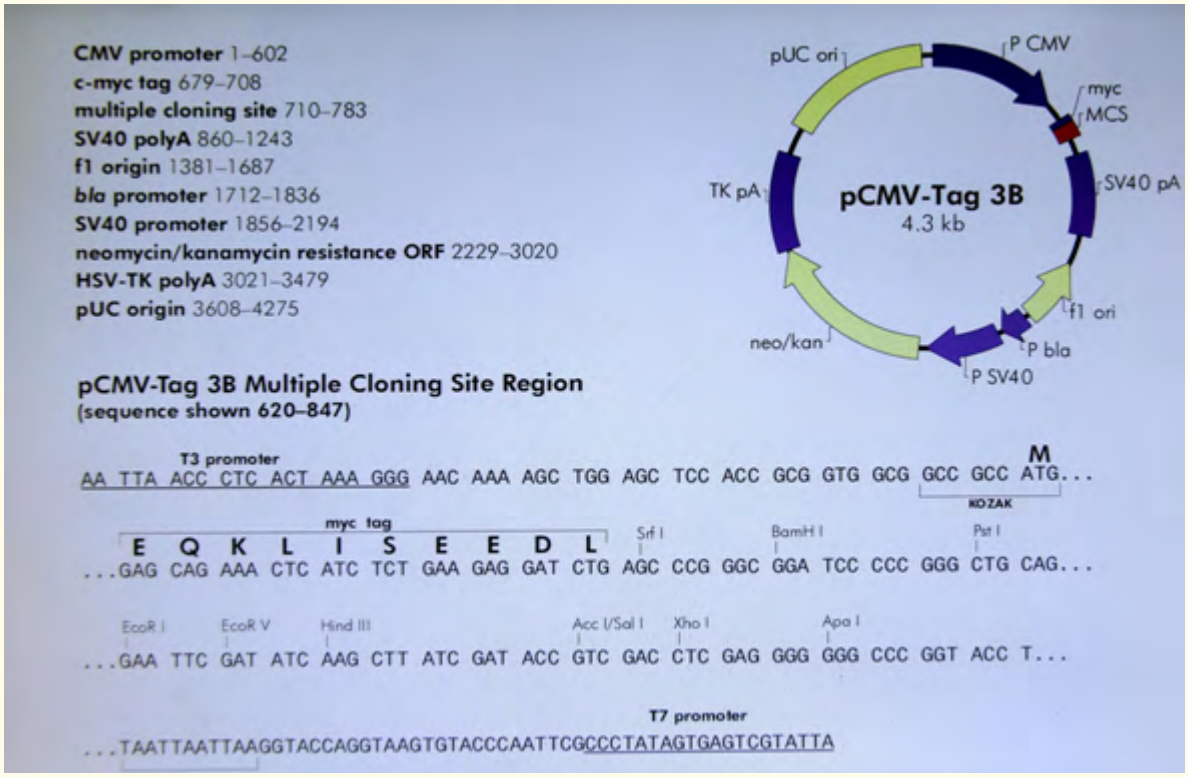

Fig. 1 and 2: The pCMV-Tag 3B.

Results

1. In 50% of observed cases, human skin cancer heterotransplantation was rejected by axial organ one in nude mouse: Extra-blood vessels were observed between the 2 heterotransplantations under the skin of the nude mouse. An antiserum (anti A.O cells) labeled many axial organ cells which were surrounding cancerous human cells in the concerning heterotransplantation
2. In 30% of observed cases, A.O cells exert an induced and spontaneous cytotoxicity against Mouse SP2 cells [2].
3. The protein « young », also named: invertebrate primitive antibody exert a spontaneous cytotoxicity 24 hours after transfection against A-375 melanome cells, U2oS cells and Hela cells. Percentages are expressed in fig. 3.

LYSIS:	A375	U2oS	Hela
	100%	100%	70%

Fig. 3: Lethality percentage, 24 hours after electroporation.

Western blots do not confirm, the protein expression because of the high lethality of cancerous cells which is obtained (The peak of the protein in western blots would be situated at 12.000 daltons).

Controls: A-375 cells, U2oS, Hela cells treated with alone electroporation show a weak lethality.

Conclusion

These results are of particular importance and show undoubtly that axial organ cells from the Asterias rubens lymphoïd organ exert a spontaneous cytotoxicity and in certain cases, an induced cytotoxicity against Cancerous Vertebrate cells.

References

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