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Short Communication

Bivalent Metal Paramagnetics to Suppress the DNA Polymerase Beta in Human Retinoblastoma Cells. A Caution

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Abstract

Nuclear spin possessing isotopes (^{25}Mg, ^{43}Ca, ^{67}Zn) promote the marked magnetic isotope effects (MIE) on DNA Polymerase Beta (DNApolB) in *ex vivo* survived human retinoblastoma (RB) cells. In Aphidicolin chase experiment, the RB *in situ* catalytic activity of DNApolB has been selectively estimated as a function of MIE. A resulted enzyme function breakdown leads to a sharp decrease of cancer cell viability. This study a paramagnetic chemotherapy path is all about.

Keywords: DNA Polymerase Beta, Aphidicolin, retinoblastoma, paramagnetic

Introduction

Some nearly prophetic predictions regarding a therapeutic validity of epigenic DNA expression control in retinoblastoma (RB) has been publicized lately [1] and then confirmed considering the cancer DNA repair machinery as a target once its key part, DNA Polymerase Beta (DNApolB, EC 2.7.7.7), overexpressed [2,3].

However, being predominantly based on experiments with RB cell lines like WERI-RB or Y79, this statement still may be treated as "immature" since the *in vivo* operating neuro-immune and neuro-endocrine paths are no doubt capable of making an impact on intracellular epigenetic environment.

This means a necessity to verify the DNApolB related findings on a donor derived RB specimen. To fit this requirement, we have investigated both cell viability and the DNApolB function in a departed patient's RB cells in culture subjected to such DNApolB effectors as the paramagnetic stable metal isotopes [3, 4].

Materials and Methods

Reagents used.

Aphidicolin (Fluka GmbH, Switzerland), AccuPrep DNA extraction kit (Bioneer Corp., Rep. Korea), [Methyl-1,2-^{3}H] dTTP, 160 -180 Ci/mmol (NEN Inc., USA), DMEM/F12 - 10%FBS cell culture medium (Gibco BRL Co., USA), chloride salts of 98.4 -99.4% pure magnetic (^{25}Mg, ^{43}Ca, ^{67}Zn) and non-magnetic (^{24}Mg, ^{40}Ca, ^{64}Zn) isotopes (Gamma Lab AS, Spain).

Donor

3.5-year-old male with RB, stage 2A. Unilateral solitary 12.5 mm tumor located within the eyeball, behind the equator line. No regional metastases found. RB diagnosis was issued three months prior to the deadly car accident at the very same clinical facility where the corps was delivered to, Moscow Region Research Clinical Institute (MRRCI). RB diagnosis background: NMR, ultrasound test, CT. No chemotherapy done. Cause of death: massive complex abdominal trauma. No eye injury. Enucleation was carried out 8 hours after the death upon a signed parental assent in accordance with an Article 47 of the Russian Federal Law # 323-FZ of 01.01.2017 "On Protection of Health of Citizens of The Russian Federation".

Experimental Procedures.

A removal of tumor and the following cells isolation/cultivation was performed as described in [5]. MTT test and a laser confocal microscopy were engaged. 12 hrs after the culture started, Aphidicolin was added to 5.0 µL/mL. 6 hrs later, the DNA labeling with [^{3}H] dTTP (90 - 110 µCi/mL) has been initiated. All cell culture samples, initially possessing isotope -pure 20 mM $MeCl_2$, were taken for a main test at +37°C, O°C parallels -controls. For protein measurements and DNA extraction, the cell lysates were prepared by Triton X-100 adjusting to 2.0%, v/v [3]. To analyze the nascent [^{3}H] DNA chains, a standard 1.8% agarose gel electrophoresis technique supplemented with ethidium bromide and autoradiography treatments has been employed [3,5]. The DNApolB catalytic activ-

ity values were expressed in ([^{3}H] DNAcpm/mg protein); note: all other representatives of the whole DNA Polymerases diversity (alpha, gamma, delta, epsilon) were selectively turned off by Aphidicolin [3].

Results and Discussion

As seen from our data, the good shape RB cells (Figure 1) are sensitive to MIE -promoting ions (Figure 2).

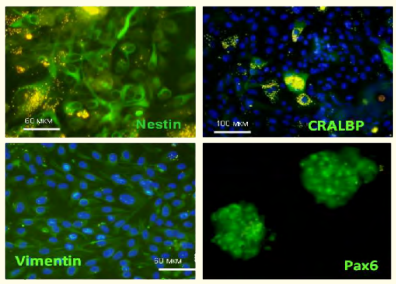

Figure 1: The Tissue Specific Markers Expressed In 72hr-Cultured Retinoblastoma Cells.
Confocal cell images visualized with Pax6, CRALBP, Nestin and Vimentin [1, 5], 20 mM $^{24}MgCl_2$.

An ion -radical mechanism of MIE [3] explain why DNApolB species are the only one's sensitive to MIE unlike all other members of a variable DNA Polymerases superfamily. Thus, in catalytic sites of the latters, the electron transfer distance (-P-O$^-$ - - - - - Me^{2+}) is too long to allow the "magnet" to express its MIE [2-4].

Noteworthy, the MIE had caused not only the DNApolB activity breakdown but an essential decrease of the resulted DNA chain lengths as well (Figure 2). This is a first report ever on the MIE-triggered production of the "size invalid" (20% to 30% shorted) DNA sequences programmed by DNApolB in cancer cells. In this case, to get smaller is likely to become insufficient to take part in cancer DNA repair and, therefore, to be incapable to contribute to survival of proliferating malignant tissue. What we've got with the MIE-related LC_{50} reflections (Figure 2), is certainly in a favor to this statement. Low contents of endogenous Fe^{2+} in neural and epithelial cells makes the MIE processes easy-to -launch [3], while the bivalent metal "magnets" might be delivered right to the cancer target by low toxic amphiphilic nanocationites [3,4]. Besides, the enrichment of a total magnesium pool with magnetic ^{25}Mg isotope may change abundant drug form in a way to optimize chemotherapy in those cases (RB including) where the MIE is expected to provide an anti -cancer result.

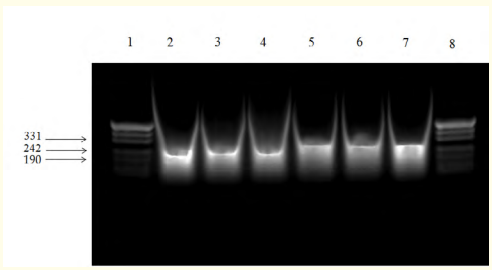

Figure 2: Agarose Gel Electrophoretic Patterns of the Nascent DNA Chains Processed by Aphidicolin Chased Retinoblastoma Cells in the Presence of Magnetic ($^{25}Mg^{2+}$, $^{43}Ca^{2+}$, $^{67}Zn^{2+}$) and Non-Magnetic ($^{24}Mg^{2+}$, $^{40}Ca^{2+}$, $^{64}Zn^{2+}$) Isotopes.

1, 8 -DNA marker tracks.
2 -20 mM (^{25}Mg) Cl_2: 18,340 [^{3}H] DNAcpm/mg protein; LC_{50} = 5.60 µg/mL.
3 -20 mM (^{43}Ca) Cl_2: 23,644 [^{3}H] DNAcpm/mg protein; LC_{50} = 3.85 µg/mL.
4 -20 mM (^{67}Zn) Cl_2: 16,080 [^{3}H] DNAcpm/mg protein; LC_{50} = 6.77 µg/mL.
5 -20 mM (^{24}Mg) Cl_2: 52,110 [^{3}H] DNAcpm/mg protein; LC_{50} = 98.36 µg/mL.
6 -20 mM (^{40}Ca) Cl_2: 49,228 [^{3}H] DNAcpm/mg protein; LC_{50} = 141.27 µg/mL.
7 -20 mM (^{64}Zn) Cl_2: 41,408 [^{3}H] DNAcpm/mg protein; LC_{50} = 183.74 µg/mL.

A trend making approach is there. Based on some previous achievements dealing with the MIE anti -cancer potential [2-4] as well as on our present data, an upgraded preclinical platform suitable for a current retinoblastoma trial is now proposed (Figure 3).

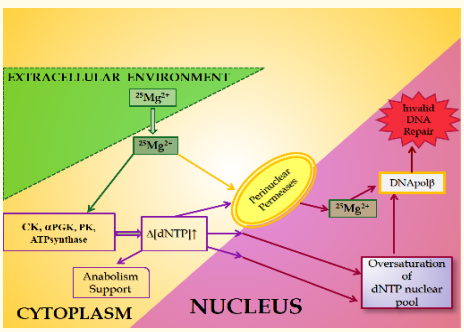

Figure 3: Magnetic Isotope Effects to Upgrade a Preclinical Research Platform for Retinoblastoma Chemotherapy Strategies.

Taking into account a reliable mode of the nanocationite providing targeted delivery of bivalent metal cations to rapidly growing tumors *in vitro* [4,6] and *in vivo* [6-8], our data presented have a clear pharmacological potential.

Conclusion

Stable paramagnetic isotopes of Mg, Ca and Zn are found of being capable to promote the essential inhibitory effects on DNA polymerase Beta in human retinoblastoma cells. This leads to a sharp decrease of cancer cells viability which might be beneficial for further use in an ongoing preclinical trial program.

Bibliography

1. Zhang J., *et al.* "A novel retinoblastoma therapy from genomic and epigenic analyses". *Nature* 48 (2012): 329-334.

2. Bozic I. and Nowak MA. "Resisting resistance". *Annual Review of Cancer Biology* 1 (2017): 203-221.

3. Bukhvostov AA., *et al.* "A new platform for anti-cancer experimental pharmacology: the DNA repair enzyme affected". *British Journal of Pharmacology and Toxicology* 5 (2014): 35-41.

4. Orlova MA., *et al.* "Effect of ^{67}Zn -nanoparticles on leukemic cells and normal lymphocytes". British *Journal of Medicine and Medical Research* 2 (2012): 21-30.

5. Kuznetsova AV., *et al.* "Method for preparation of culture of the human retina pigment epithelium cells". *Russian Ophthalmology* (in Russian) 1(2011): 244-254.

6. Kuznetsov DA., *et al.* "A non-Markovian population dynamics in the chemotherapy strategy decision making". *Journal of Biomedical Sciences* 6 (2010): 100-110.

7. Buchachenko AL. "Why magnetic and electromagnetic effects in biology are ambiguous and contradictory?". *Bioelectromagnetics* 37 (2016): 1-13.

8. Buchachenko AL and Lowler R. "New possibilities in magnetic control of chemical and biochemical reactions". *Accounts of Chemical Research* 50 (2017): 977-884.

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