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Methotrexate-resistance is associated with double minute chromosomes in lymphocytes from patients with prostate cancer

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ارتباط المقاومة للميثوتركسيت في لمفاويات الم المحيطي لمرضى سرطان البروستات مع المكروسومات الدقيقة المضاعفة

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الخلاصة

تستعمل الخلايا اللمفاوية منذ مدة طويلة لتقييم الضرر الجيني الذي قد يحدث داخل الجسم الحي وخارجه. في هذه الدراسة،زرعت لمفاويات الدم المحيطي المأخوذة من المرضى المصابين بسرطان البروستات في المزروع القصير الأمد بوجود عقار الميثوتركسيت MTX خارج الجسم الحي لبحث الإلية المحتملة لمقاومة هذه اللمفاويات للميثوتركسيت . تشير نتائج هذه الدراسه الى ان مقاومة لمفاويات الدم المحيطي الماخوذه من مرضى سرطان البروستات لعقار الميثوتركسيت ترتبط مع تضخم الجين داي هيدروفوليت-ريكتيز *DHFR* . واختبرت أيضا كروموسومات الطور الاستوائي للخلايا اللمفاوية قيد الدراسة ولوحظ أنها تحتوي على الكروموسومات الدقيقة المضاعفة . وبناء على نتائج الدراسة نستطيع الاستنتاج إن وجود الكروموسومات الدقيقة المضاعفة يدعم الارتباط بين مقاومة هذه الخلايا لعقار الميثوتركسيت والتضخم في جين الداي هيدروفوليت-ريديكتيز.

Abstract

Human lymphocytes have long been used to assess the gene damage occurring *in vivo* as well as *in vitro* . In this study , peripheral blood lymphocytes from patients with prostate cancer were cultivated *in vitro* short-term culture in the presence of methotrexate (MTX) to investigate the possible mechanism of resistance to MTX in those cells. The results indicate that the resistance to MTX in lymphocytes from prostate cancer patients is associated with *dihydrofolate reductase (DHFR)* gene amplification . Metaphase chromosome preparations from those cells were examined and were shown to contain double minute chromosomes (DMs). We conclude, that the presence of

DMs may support that the resistance to MTX in prostate cancer patients may be associated with *DHFR* gene amplification.

Introduction

Mammalian cells have been shown to acquire resistance to a dihydrofolate reductase (DHFR) inhibitor,methotrexate (MTX) , (a 4-amin analog of folic acid) , either through an amplification of the *DHFR* gene , which leads to overproduction of the target DHFR enzyme [1,2] , through mutations rendering the DHFR enzyme less sensitive to MTX , or through mutations decreasing MTX uptake by cells. Of these causes of the most frequent one under a variety of experimental conditions [3,4]. Amplified genes have been associated with two types of microscopically visible aberrant chromosome structures: expanded chromosomal regions , referred to as homogeneously staining regions (HSRs)[5] , and extrachromosmal acentric fragments , referred to as double minute chromosomes (DMs)[6]. Methotrexate resistance murine cell lines usually in DMs , whereas Chinese and Syrian hamster cells virtually always maintain DHFR (and all other) amplicons in HSRs [7, 8,9]. In human [10,11] and rat cell lines , DHFR amplicons can be manifested either as HSRs or DMs , although in human tumor samples , they most often appear as DMs[12].

Prostate cancer is a complex disease and the most generally accepted model of carcinogenesis postulates that cancer develops through the accumulation of genetic alternations that allow the cells to escape normal growth regulatory mechanisms [13]. Human lymphocytes have long been used to assess genetic damage occurring *in vivo* as well as *in vitro*. *DHFR* gene is an important biomarker of effect genome-mutational events [14]. We use *in vitro* lymphocyte culture to gain insight into the possible mechanism of resistance to MTX of peripheral blood lymphocytes from patients with prostate cancer.

Material and methods

Thirteen men with prostate cancer were the subjects of this study. Short-term peripheral blood lymphocyte cultures were performed under optimal conditions [15]. In brief : 0.25 ml of heparainized blood was inoculated in 2 ml of RPMI-1640 medium (Serva) containing 0.2 ml of phytohemagglutinin (PHA) was obtained as a stock solution prepared by directorate of Hazardous materials/Ministry of Science and Technology-Baghdad-Iraq, and 10% fetal calf serum (sigma).MTX (Oncohexa) was prepared in a concentration of

4mg/ml which was added to peripheral blood lymphocyte cultures for each sample of patients to determine the resistant cells.

The cultures were manipulated in 10 ml sterile vacutainer tube and then incubated at 37C for 72 hrs. The harvesting of mitogen-stimulated cells begins with adding 0.1 ml f colchicine (Serva) in a concentration of 10 mg/ml to each culture tube for the final two hours of incubation time. The cells treated for 20 min with the hypotonic solution (0.075 M kcl) and fixed with methanol: acetic acid (3:1 vol/vol). Cells were dropped onto clean slides. Metaphases were stained with Giemsa solution (2.5%) (Fluka) for 30 min.

Results and Discussions

It is well-established that the cytogenetic analysis of peripheral blood lymphocyte culture constitutes an important and sensitive tool in a wide range of studies designed to ascertain the genetic effects in several disease .In this study, lymphocytes obtained from patients with prostate cancer that treated with MTX *in vitro* showed marked resistance which represented by the ability of these cells to form blasts and dividing in the presence of the cytotoxic drug. Furthermore, MTX-resistant lymphocytes from prostate cancer patients were examined for the presence of double minute chromosomes. Figure 1 shows photomicrograph of MTX –resistance lymphocyte from those patients which have large numbers of double minute chromosomes.

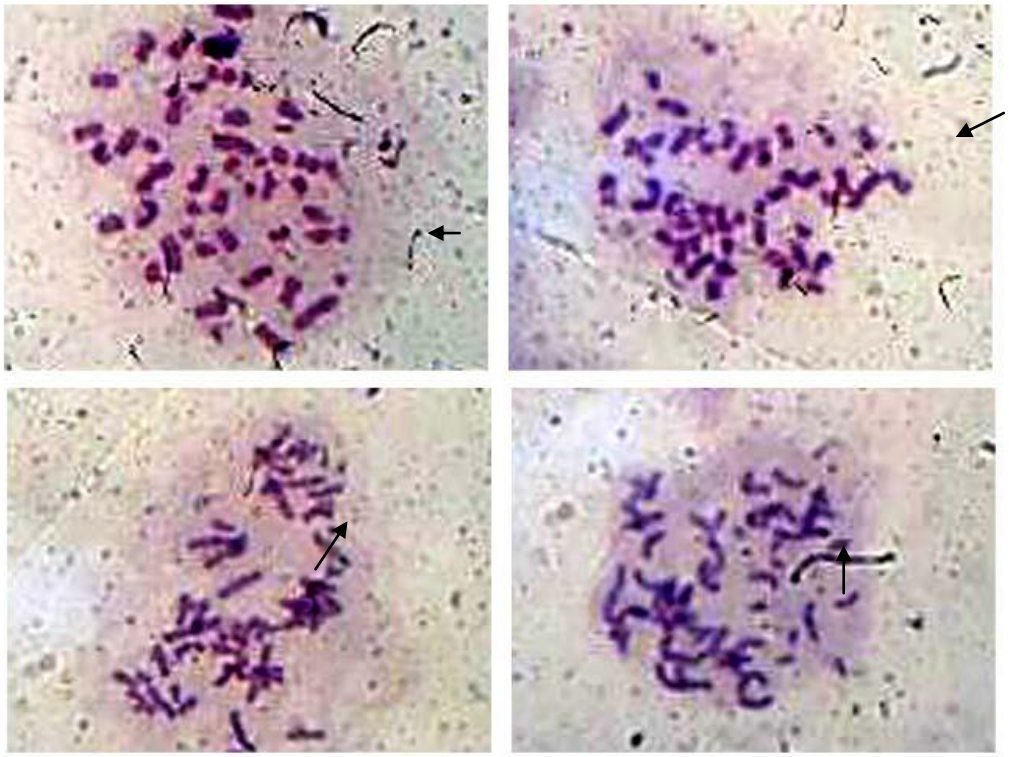

Fig .1. Double minute chromosomes in the MTX-resistant cells from patients with prostate cancer.

Genomic amplifications are mutations associated with drug resistance and tumor progression in mammalian cells [9 , 16]. Amplification results from chromosomal rearrangements that lead to multiple gene copies and overproduction of the product of the amplified gene. The amplification of the *DHFR* gene in Chinese hamster cells is initiated by chromosome breaks, followed by bridge-breakage-fusion cycles that generate large intrachromosomal repeats; these are trimmed by an unknown process to smaller, more homogeneous units (HSRs). However: in most human tumor cells, amplified DNA sequences are borne on unstable, extrachromosomal double minutes (DMs), which suggest the operation of a different amplification mechanism [17].

In this work, we describe that resistance to MTX of lymphocytes from patients with prostate cancer is associated with amplification of *DHFR* gene via the presence of double minute chromosomes. Kaufman *et al* (1979) indicated that unstably amplified DHFR genes are associated with DMs are the following: (i) Double minutes are present in cell lines with unstably amplified *DHFR* genes. (ii) As unstably MTX-resistant cells are propagated in the absence of MTX, the amplified double *DHFR* genes and the double minutes are lost. (iii) Double minutes appear with the development of unstable MTX-resistant. (iv) When unstable MTX-resistant cells become stably resistant, double minutes disappear. Singer and his co-workers isolated a large number of independent MTX-resistant human tumor cell lines, all of which contained *DHFR*-bearing DMs , suggesting that DMs are initiated by chromosomal breaks. The initial event in the generation of DMs may involve disproportionate chromosomal replication and subsequent excision-replication [18]. Conversely, DNA may be taken up by lysis of cells and subsequent replication of DNA containing the *DHFR* sequences [6].

In conclusion, the resistance to MTX in lymphocytes from patients with prostate cancer is accounting for the amplification of *DHFR* gene. This is confirmed by the presence of double minute chromosome.

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