

DOXORUBICIN AND ETOPOSIDE INDUCE SOMATIC RECOMBINATION IN DIPLOID CELLS OF *ASPERGILLUS NIDULANS*

Simone Jurema Ruggeri Chiuchetta, Marialba Avezum Alves de Castro-Prado

Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Maringá, Paraná, Brasil.

Submitted: May 27, 2002; Approved: September 24, 2002

ABSTRACT

Doxorubicin and etoposide are intercalating agents that inhibit the action of the enzyme topoisomerase II. Both drugs present therapeutic activity in numerous human neoplasms. In the present work the recombinogenic potential of these drugs was evaluated by the ascomycete *Aspergillus nidulans*. Their effects on the asexual cycle of *A. nidulans* was also appraised. Two heterozygous diploid strains of *A. nidulans*, a wild (*uvsH+//uvsH+*) and a defective to the DNA repair (*uvsH//uvsH*) were used. The drugs' recombinogenic potential was evaluated by their capacity to induce homozygosity of recessive genes from heterozygous cells. Both drugs have a recombinogenic effect on diploid cells of *A. nidulans*. Doxorubicin and etoposide are potentially capable to induce secondary malignancies, mediated by the mitotic crossing-over in eukaryotic cells.

Key words: antineoplastic agents, mitotic crossing-over, secondary malignancies

INTRODUCTION

Doxorubicin and etoposide are antineoplastic drugs classified as intercalating agents, a cytotoxic group of drugs that, bound to DNA, inhibits the action of topoisomerase II (11).

Doxorubicin is an anthracycline antibiotic produced by fungus *Streptomyces peucelii* var. *caesius*; etoposide is a semi-synthetic glycoside from podophyllotoxin, an active compound extracted from the *Podophyllum peltatum* plant. Both drugs present therapeutic activity in numerous human neoplasms such as breast cancer, ovary carcinoma, head and neck carcinoma, leukemia, lung carcinoma, and testicle tumors (5).

Studies on human fibroblasts demonstrated that the two intercalating agents, doxorubicin and etoposide, block the S phase of the cellular cycle after a short period of cell treatment (15). An induction of breaks in DNA double-strands by doxorubicin and etoposide was also reported in leukemia cells after 3 hrs of exposition to the chemotherapeutic agents (8).

Chromosomal breaks and high frequencies of somatic recombination have been observed in cells of Bloom's syndrome or Fanconi anemia patients whose present predisposition to cancer development. Due to an exchange of segments between

homologous chromatids, the somatic recombination seems to be associated with the high cancer incidence reported in these diseases (7,9,17).

Two chromosomal events are involved in the development of neoplasms: i) a genetic or epigenetic alteration that results in a pre-malignant heterozygous condition (*m/+*) and ii) a chromosomal rearrangement involving the affected locus, which results in homozygosity (*m/m*) or hemizygosity (*m/0*), with the subsequent expression of the recessive malign character. Homozygous cells (*m/m*) may originate from the exchange of segments between homologous chromatids, in the G2 phase of the cellular cycle, preceded by the segregation of recombinant chromatids to opposite mitotic poles (3,14).

The ascomycete *Aspergillus nidulans* is an excellent system for the study of mitotic crossing-over. This is due to the fact that its cells pass a substantial part of their cellular cycle in the G2 phase during germination. At this phase, the existence of two copies of each chromosome benefits mitotic recombination events (12).

Our research aimed at investigating the roles of doxorubicin and etoposide in the homologous interchromosomal recombination in heterozygous cells of *A. nidulans*. Two diploid strains were used, a defective to DNA repair and a normal one.

* Corresponding author. Mailing address: Universidade Estadual de Maringá, Departamento de Biologia Celular e Genética. Av. Colombo, 5790. 87020-900, Maringá, PR, Brasil. Tel.: (+5544) 261-4679. E-mail: maacprado@uem.br

MATERIALS AND METHODS

Strains

The *A. nidulans* strains were derived from Utrecht (UT448) and FGSC (A757, A737). The B211 strain was obtained in our laboratory (4). Diploid strains (UT448//A757 and B211//A837) were prepared according to Roper (16). Genotypes of the strains: a) UT448: *riboA1*, *pabaA124*, *biA1*, *AcrA1*, *wA2*; b) A757: *yA2*, *methA17*, *pyroA4*; c) A837: *pabaA1*, *UvsH77*, *pyroA4*, *choA1*, *chaA1*; d) B211: *yA2*, *biA1*, *AcrA1*, *wA2*, *methA17*, *UvsH77*, *pyroA4*, *chaA1*.

Culture media

Complete medium (CM) and minimum medium (MM) were prepared as described by Van de Vate and Jansen (19). The selective medium (SM) was prepared with MM supplemented with the nutritional requirements for each strain. Solid medium was prepared with 1.5 % agar and strain growth was incubated at 37°C.

Doxorubicin and etoposide treatment

Doxorubicin (ICN), dissolved in an aqueous solution, and etoposide (ICN), dissolved in 10µl DMSO, were added to molten MM. Solvent was *per se* neither visibly toxic nor recombinogenic for the diploid strain (results not shown). Doxorubicin concentration that induced breaks in DNA strand in leukemia cells at 2.0µg/ml (8) and etoposide concentration with induced cell cycle arrested in normal fibroblast at 1.0 µg/ml (15) and at higher concentrations (4.0µg/ml doxorubicin and 3.0µg/ml etoposide) were used in the present study. They were final in agar medium.

Evaluation of the recombinogenic potential

Conidia of each diploid strain were inoculated in MM + antineoplastic agents. Treatment produced visible diploid sectors, D1-D32, identified by their different morphology from the original diploid. Diploid sectors were submitted to spontaneous haploidization in CM after purification in MM. Only haploid segregants were selected for recombinogenesis test. Conidia of each haploid sector were transferred to 25 defined positions in CM plates (master plates). After 48 hours of incubation at 37°C, colonies were transferred to SM and the phenotypical analysis of the haploid segregants was carried out.

The treatment with the drugs in MM will produce only heterozygous (+ / - or - /+) or homozygous (+/+) segregants because the recessive ones (-/-) fail to grow in MM. After haploidization of diploids D1-D32 the nutritional markers will segregate among the haploids in the proportion of 4+: 4-, if drug fails to induce recombinogenesis; or 4+: 2-, if drug induces crossing-over. Values of Homozigotization Indexes (HI) (the ratio between number of phototropic and auxotrophic segregants) equal or above 2.0 evidence the recombinogenic effects of the

substance under analysis (2,13). Results were compared by Yates correct Chi-Square test.

Cytological analysis

Colonies of UT448//A757 diploid were cultivated in dialysis membranes aseptically placed on the surface of plates containing CM and CM + doxorubicin or etoposide. Samples were collected after 8, 12, 18 and 24 hours of incubation at 37°C. Membranes were stained with lactophenol cotton blue and examined under an optic microscope.

RESULTS

The mycelial growth of UT448//A757 and B211//A837 diploid strains cultivated in MC + doxorubicin (2.0 and 4.0µg/ml) and etoposide (1.0 and 3.0µg/ml) was normal when compared with that of controls (results not shown). On the other hand, alterations in the morphology of the conidiophores were observed in the cytological preparations of the UT448//A757 diploid, cultivated in the presence of the two antineoplastic agents. Conidiophore is formed by an aerial hypha, a multinucleated vesicle and two tiers of uninucleate cells (metulae and phialides) forming the sterigmata (18). Vacuoles in the stalk and in the conidiophore's vesicle were observed in preparations with 2.0µg/ml and 1.0µg/ml of doxorubicin and etoposide respectively (data not given). Conidiophores with shortened and malformed stalks were observed in higher doses of each drug (4.0µg/ml of doxorubicin and 3.0µg/ml of etoposide) (Fig. 1).

Methodology does not permit the isolation of auxotrophic diploids (-/-) since they are not selected in MM. However, homozygous recessive diploids may be obtained for conidia coloration markers (*y*, *w* and *cha*). The treatment of B211//A837 diploid strain with both drugs merely allowed the isolation of prototrophic diploids with *chartreuse* (*cha//cha*) conidia. Contrastingly the treatment with doxorubicin of UT448//A757 diploid originated prototrophic diploids with green (*y+//y*) and yellow (*y//y*) conidia. Through the phenotypical analyses of D8 (yellow) it was possible to determine that it is recombinant for the centromere-*paba* interval. The diploid D8 showed HI value higher than 2.0 for the *meth* marker (Table 1).

Most diploids from UT448//A757 strain exhibited HIs <2.0 for doxorubicin and etoposide. HI value of only one diploid was higher than 2.0 for doxorubicin (2.0µg/ml) and etoposide (1.0µg/ml), and the markers involved were *meth* and *bi* respectively. In higher concentrations the recombinogenic effect was reported in markers *paba* and *meth* for doxorubicin, and in *paba* and *bi* for etoposide (Tables 1 and 2).

Diploids from B211//A837 strain exhibited the highest sensitivity for antineoplastic. This fact was confirmed from high values of HI obtained in the recombinogenic test. For both doses of each drug, the markers involved in the recombinogenic effect were *paba* and *bi* (Tables 3 and 4). Analyses also determined

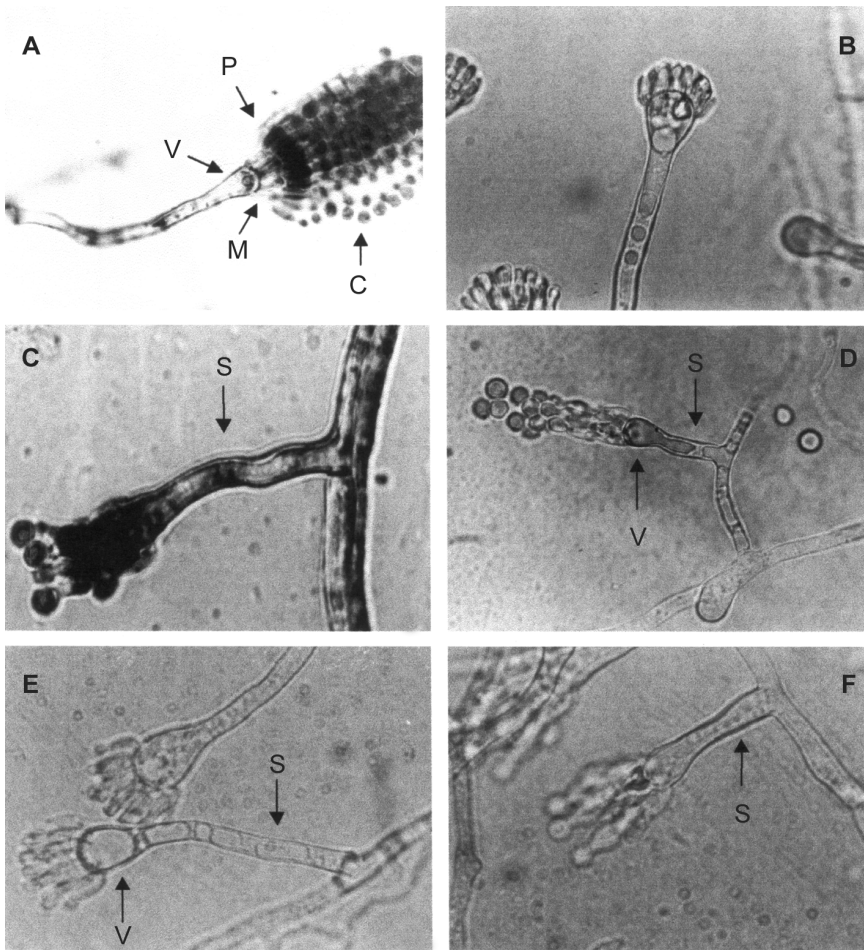


Figure 1. Growth of the conidiophore UT448//A757 in MC and in MC + antineoplastics agents. A) normal conidiophore; B) presence of vacuole in the conidiophore's vesicle (doxorubicin 4.0µg/ml); C) shortened and malformed conidiophore stalk (doxorubicin 4.0µg/ml); D) presence of vacuole in the vesicle and shortened conidiophore stalk (doxorubicin 4.0µg/ml); E) presence of vacuole in the vesicle and in the conidiophore stalk (etoposide 3.0µg/ml); F) shortened conidiophore stalk (etoposide 3.0µg/ml); V: vesicle; M: metulae; P: phialides; C: conidium; S: conidiophore stalk. Diameter of the conidiophore's vesicle corresponds to 10µm.

that diploids from B211//A837 strain produced a high number of haploid mitotic segregants in MC.

DISCUSSION

Alterations in DNA caused by chemical substances may act as starters in a complex carcinogenesis process. The loss of heterozygosity in normal cells, which carry mutations in malignant genes, by mitotic recombination, may unchain a neoplastic process (14).

In Fanconi's anemia and in Bloom's syndrome, cells of affected individuals present an enhanced level of chromosome breakage, somatic recombination and an increase in neoplastic incidence. The risk of developing tumors in these individuals is nearly 15,000-fold greater than that of the general population. The loss of heterozygosity in specific genes through the recombination mechanism in somatic cells may explain the increase of this incidence. It even corroborates the hypothesis that cancer may be a recessive disease at cell level (7,9,17).

Mycelial growth of UT448//A757 and B211//A837 diploids in the presence of doxorubicin and etoposide was similar to that of controls (results not shown). Results indicate that doses of the antineoplastic agents used in current research do not provide any toxicity to the organism under analysis, although alterations in the structure of the conidiophore (asexual cycle) were microscopically observed (Figure 1).

Table 1. Homozygotization Index (HI) of UT448//A757 diploid strain exposed to 2.0µg/ml (D1-D4) and to 4.0µg/ml of doxorubicin (D1-D8). n° seg.; number of haploid mitotic segregants. C., control.

| | C | | D1 | | D2 | | D3 | | D4 | | D5 | | D6 | | D7 | | D8 | |
|--------------|---------|------|---------|------|---------|------|---------|------|---------|-------|---------|------|---------|------|---------|-------|---------|-------|
| | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI |
| <i>paba+</i> | 37 | 1.15 | 39 | 1.50 | 45 | 1.55 | 40 | 1.82 | 54 | 1.92 | 42 | 1.62 | 47 | 1.88 | 45 | *2.14 | 72 | 0 |
| <i>paba-</i> | 32 | | 26 | | 29 | | 22 | | 28 | | 26 | | 25 | | 21 | | 0 | |
| <i>bi+</i> | 40 | 1.37 | 36 | 1.24 | 41 | 1.24 | 37 | 1.48 | 52 | 1.73 | 41 | 1.52 | 42 | 1.40 | 42 | 1.75 | 72 | 0 |
| <i>bi-</i> | 29 | | 29 | | 33 | | 25 | | 30 | | 27 | | 30 | | 24 | | 0 | |
| <i>meth+</i> | 38 | 1.22 | 41 | 1.70 | 49 | 1.96 | 39 | 1.69 | 56 | *2.15 | 41 | 1.52 | 43 | 1.48 | 40 | 1.53 | 49 | *2.13 |
| <i>meth-</i> | 31 | | 24 | | 25 | | 23 | | 26 | | 27 | | 29 | | 26 | | 23 | |

* Significantly different from control, $P < 0.05$ (Yates correct Chi-square test, Statistic for Windows Program).

Table 2. Homozygotization Index (HI) of UT448//A757 diploid strain exposed to 1.0µg/ml (D9-D12) and to 3.0µg/ml of etoposide (D13-D16). n° seg.; number of haploid mitotic segregants. C., control.

| | C | | D9 | | D10 | | D11 | | D12 | | D13 | | D14 | | D15 | | D16 | |
|--------------|---------|------|---------|-------|---------|------|---------|------|---------|------|---------|-------|---------|------|---------|------|---------|-------|
| | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI |
| <i>paba+</i> | 37 | 1.15 | 43 | 1.72 | 37 | 1.85 | 35 | 1.40 | 47 | 1.80 | 42 | *2.33 | 40 | 1.90 | 44 | 1.57 | 35 | 1.66 |
| <i>paba-</i> | 32 | | 25 | | 20 | | 25 | | 26 | | 18 | | 21 | | 28 | | 21 | |
| <i>bi+</i> | 40 | 1.37 | 46 | *2.09 | 32 | 1.28 | 39 | 1.85 | 46 | 1.70 | 34 | 1.30 | 31 | 1.03 | 42 | 1.40 | 39 | *2.29 |
| <i>bi-</i> | 29 | | 22 | | 25 | | 21 | | 27 | | 26 | | 30 | | 30 | | 17 | |
| <i>meth+</i> | 38 | 1.22 | 44 | 1.83 | 33 | 1.37 | 38 | 1.72 | 43 | 1.43 | 35 | 1.40 | 39 | 1.77 | 46 | 1.76 | 34 | 1.54 |
| <i>meth-</i> | 31 | | 24 | | 24 | | 22 | | 30 | | 25 | | 22 | | 26 | | 22 | |

* Significantly different from control, P<0.05 (Yates correct Chi-square test, Statistic for Windows Program).

Table 3. Homozygotization Index (HI) of the B211//A837 diploid strain exposed to 2.0µg/ml (D17-D20) and to 4.0µg/ml of doxorubicin (D21-D24). n° seg.; number of haploid mitotic segregants. C., control.

| | C | | D17 | | D18 | | D19 | | D20 | | D21 | | D22 | | D23 | | D24 | |
|--------------|---------|------|---------|------|---------|-------|---------|-------|---------|------|---------|------|---------|-------|---------|-------|---------|-------|
| | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI |
| <i>paba+</i> | 73 | 1.97 | 78 | 2.51 | 64 | 1.82 | 82 | *3.15 | 61 | 1.52 | 65 | 1.85 | 62 | 1.93 | 76 | *4.00 | 66 | 1.69 |
| <i>paba-</i> | 37 | | 31 | | 35 | | 26 | | 40 | | 35 | | 32 | | 19 | | 39 | |
| <i>bi+</i> | 70 | 1.75 | 67 | 1.59 | 76 | *3.30 | 68 | 1.70 | 57 | 1.29 | 61 | 1.56 | 67 | *2.48 | 62 | 1.87 | 80 | *3.20 |
| <i>bi-</i> | 40 | | 42 | | 23 | | 40 | | 44 | | 39 | | 27 | | 33 | | 25 | |
| <i>meth+</i> | 72 | 1.89 | 45 | 0.70 | 52 | 1.10 | 56 | 1.07 | 45 | 0.80 | 30 | 0.42 | 39 | 0.70 | 42 | 0.79 | 48 | 0.84 |
| <i>meth-</i> | 38 | | 64 | | 47 | | 52 | | 56 | | 70 | | 55 | | 53 | | 57 | |

* Significantly different from control, P<0.05 (Yates correct Chi-square test, Statistic for Windows Program).

Table 4. Homozygotization Index (HI) of the B211//A837 diploid strain exposed to 1.0µg/ml (D25-D28) and to 3.0µg/ml of etoposide (D29-D32). n° seg.; number of haploid mitotic segregants. C., control.

| | C | | D17 | | D18 | | D19 | | D20 | | D21 | | D22 | | D23 | | D24 | |
|--------------|---------|------|---------|-------|---------|------|---------|-------|---------|-------|---------|-------|---------|------|---------|-------|---------|-------|
| | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI | n° seg. | HI |
| <i>paba+</i> | 73 | 1.97 | 76 | *3.04 | 69 | 1.72 | 75 | 2.14 | 82 | *3.56 | 81 | *3.68 | 65 | 1.75 | 78 | *3.90 | 88 | *4.00 |
| <i>paba-</i> | 37 | | 25 | | 40 | | 35 | | 23 | | 22 | | 37 | | 20 | | 22 | |
| <i>bi+</i> | 70 | 1.75 | 65 | 1.80 | 72 | 1.94 | 83 | *3.07 | 69 | 1.91 | 67 | 1.86 | 67 | 1.91 | 64 | 1.88 | 85 | *3.40 |
| <i>bi-</i> | 40 | | 36 | | 37 | | 27 | | 36 | | 36 | | 35 | | 34 | | 25 | |
| <i>meth+</i> | 72 | 1.89 | 49 | 0.94 | 57 | 1.09 | 53 | 0.92 | 57 | 1.18 | 50 | 0.94 | 54 | 1.12 | 48 | 0.96 | 58 | 1.11 |
| <i>meth-</i> | 38 | | 52 | | 52 | | 57 | | 48 | | 53 | | 48 | | 50 | | 52 | |

* Significantly different from control, P<0.05 (Yates correct Chi-square test, Statistic for Windows Program).

Concerning the recombinogenic potential, results obtained indicate that doxorubicin and etoposide were also capable of inducing homozygosis. These observations were based on the high Homozygotization Indexes values (HI > 2.0) obtained for diploids treated with the two drugs (Tables 1-4).

The fact that diploids derived from B211//A837 strain showed HI values higher than those derived from UT448//A757 one is due to the *uvr* mutation in their genome. Homozygous diploid strains for *uvrH* mutation cause an efficient increase in the mitotic crossing-over frequency and in chromosome instability (10).

The loss of a functional copy of a heterozygous tumor suppressor gene represents an important step during neoplastic transformation. (1). Treatment with an anticancer drug causing mitotic crossing-over might lead to the expression of recessive genes, previously masked in a heterozygous condition. The clinical use of these drugs might increase cancer risk (6). Our results demonstrate the carcinogenic potential of doxorubicin and etoposide antineoplastics through heterozygosity loss. They are potentially capable of inducing secondary malignancies by the mitotic crossing-over.

RESUMO

Doxorubicina e etoposida induzem a recombinação somática em células diplóides de *Aspergillus nidulans*

Doxorubicina e etoposida são agentes intercalantes que inibem a ação da enzima topoisomerase II. Ambas drogas são amplamente utilizadas no tratamento de neoplasias. O potencial recombinagênico destes agentes e seus efeitos sobre o ciclo assexual de *Aspergillus nidulans* foram avaliados no presente trabalho. Duas linhagens diplóides heterozigotas foram utilizadas: uma selvagem (*uvsH+//uvsH+*) e outra deficiente para o reparo do DNA (*uvsH//uvsH*). O potencial recombinagênico destas drogas foi avaliado pela indução de homozigose de genes recessivos a partir de células heterozigotas. Doxorubicina e etoposida apresentaram efeitos recombinagênicos em ambas linhagens utilizadas. Os resultados permitem concluir que doxorubicina e etoposida são agentes potencialmente capazes de induzir malignidades secundárias, mediadas pelo crossing-over mitótico, em células diplóides eucariotas.

Palavras-chave: agentes antineoplásicos, crossing-over mitótico, malignidade secundária.

REFERENCES

- Acuma, G.; Wurgler, F.E.; Sengstag, C. Reciprocal mitotic recombination is the predominant mechanism for the loss of a heterozygous gene in *Saccharomyces cerevisiae*. *Environ Mol Mutagen.*, 24(4): 307-316, 1994.
- Baptista, F.; Castro-Prado, M.A.A. Benlate-induced homozygosis from heterozygous diploids strain in *Aspergillus nidulans*. *Cytologia*, 62: 389-396, 1997.
- Beumer, K.J.; Pimpinelli, S.; Golic, K.G. Induced chromosomal exchange directs the segregation of recombinant chromatids in mitosis of *Drosophila*. *Genetics*, 150: 173-188, 1998.
- Busso, C.; Chiuchetta, S.J.R.; Baptista, F.; Castro-Prado, M.A.A. *uvsH//uvsH* diploid strain favors an efficient method to evaluate the recombinagenic effect of chemical and physical agents in *Aspergillus nidulans* (Ascomycetes). *Acta Scientiarum*, 23(2): 603-607, 2001.
- Chabner, B.A.; Allegra, C.J.; Curt, G.A.; Calabresi, P. Antineoplastic agents. In: Molinoff P.B. and Ruddon R.W. eds. The pharmacological basis of therapeutics. (9 ed.). New York: Mc Graw-Hill, 1233-1287, 1996.
- Ferguson, L.R.; Turner, P.M. Mitotic crossing-over by anticancer drugs in *Saccharomyces cerevisiae* strain D5. *Mutat Res.*, 204: 239-249, 1988.
- Festa, R.S.; Meadows, A.T.; Boshes, R.A. Leukemia in a black child with Bloom's syndrome : somatic recombination as a possible mechanism for neoplasia. *Cancer*, 44: 1507-1510, 1979.
- Froelich, J.J.; Schneller, F.R.; Zahn, R.K. The influence of radiation and chemotherapy-related DNA strand breaks on carcinogenesis: an evaluation. *Clin Chem Lab Med.*, 37: 403-408, 1999.
- Hickson, I.D.; Davies, S.L.; Li, J.L.; Levitt, N.C.; Mohaghegh, P.; North, P.S.; Wu, L. Role of the Bloom's syndrome helicase in maintenance of genome stability. *Biochem Soc Trans.*, 29: 201-204, 2001.
- Iwanejko, L.; Cotton, C.; Jones, G.; Tomsett, B.; Strike, P. *nuvA*, an *Aspergillus nidulans* gene involved in DNA repair and recombination, is a homologue of *Saccharomyces cerevisiae* *RAD18* and *Neurospora crassa* *uvs-2*. *Microbiology*, 142: 505-515, 1996.
- Noviello, E.; Aluigi, M.G.; Cimoli, G.; Rovini, E.; Mazzoni, A.; Parodi, S.; Sessa, F.; Russo, P. Sister-chromatid exchanges, chromosomal aberrations and cytotoxicity produced by topoisomerase II-targeted drugs in sensitive (A2780) and resistant (A2780-DX3) human ovarian cancer cells: correlations with the formation of DNA double-strand breaks. *Mut Res.*, 311: 21-29, 1994.
- Osman, F.; Tomsett, B.; Strike, P. The isolation of mutagen-sensitive *nuv* mutants of *Aspergillus nidulans* and their effects on mitotic recombination. *Genet.*, 134: 445-454, 1993.
- Pires, L.T.A.; Zucchi, T.M.A.D. A new method to detect potential genotoxic agents using mitotic crossing over in diploid strains of *Aspergillus nidulans*. *Braz. J. Genet.*, 17: 371-376, 1994.
- Radman, M.; Kinsella, A.R. Chromosomal events in carcinogenic initiation and promotion : implication for carcinogenicity testing and cancer prevention strategies. *IARC Sci. Publ.*, 27: 75-90, 1980.
- Robles, S.J.; Burchler, P.W.; Negruz, A.; Adami, G.R. Permanent cell cycle arrest in asynchronously proliferating normal human fibroblasts treated with doxorubicin or etoposide but not camptothecin. *Biochem. Pharm.*, 58: 675-685, 1999.
- Roper, J.A. Production of heterozygous in filamentous fungi. *Experientia*, 8: 14-15, 1952.
- Thyagarajan, B.; Campbell, C. Elevated homologous recombination activity in Fanconi anemia fibroblasts. *J. Biol. Chem.*, 272: 23328-23333, 1997.
- Timberlake, W.E. Molecular genetics of *Aspergillus* development. *Annu. Rev. Genet.*, 24: 5-36, 1990.
- Van de Vate, C.; Jansen, G.J.O. Meiotic recombination in a duplication strain of *Aspergillus nidulans*. *Gen Res.*, 31: 29-52, 1978.