EVOLUTION OF THE CHROMOSOME CHARACTERISTICS IN A CANINE MASTOCYTOMA IN THE COURSE OF FIVE PASSAGES

EVOLUCIÓN DE LAS CARACTERÍSTICAS CROMOSÓMICAS DE UN MASTOCITOMA CANINO EN EL CURSO DE CINCO PASES

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Palabras clave adicionales

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SUMMARY

A canine grade I mastocytoma from a 10-year-old female Braco breed dog was maintained in continuous culture for three months (5 passages). One morphological cell type is always evident, the spindle fibroblast-like cell, though after about one month of culture (passages 4-5), the cells began to show a degenerative change which is characterized by bizarre shapes with long protoplasmic processes. Cytogenetic evaluation of tumour cells showed a chromosome number ranging from less than 73 to more than 78. 70 p. cent of the cells in the explante had the modal number of chromosomes in dog (78). This chromosome number decreased throughout the passages, this number being the most frequent during the early three passages. However in the 4th and 5th passages the most frequent number was less than 73 chromosomes. The number of biarmed chromosomes also had the same behaviour as the modal number of chromosomes. During the first three passages, the modal number of biarmed chromosomes was two, the X chromosomes. In the last two passages, this modal number was dramatically increased, the most common number being more than three. In general, the more common alterations of this type of tumor were hypodiploid cells, additional biarmed chromosomes, and monosomy of X chromosome.

RESUMEN

Se ha mantenido durante 3 meses (5 pases) en cultivo continuo un mastocitoma canino de grado I de un perro hembra de raza Braco de 10 años de edad. Siempre ha sido evidente la presencia de un tipo morfológico celular, el fibroblasto en huso, que a partir del mes de cultivo muestran cambios degenerativos. La evaluación citogenética de las células tumorales mostró un número de cromosomas muy variable, con un rango de menos de 73 a más de 78. El 70 p. cien de las células de los explantes tenían el número modal del cromosoma del perro (78). Este número cromosómico decrecía a través de los distintos pa-

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sos, sin embargo en el cuarto y quinto paso el número más frecuente fue menos de 73 cromosomas. Por otra parte el número de cromosomas bibraquiales tenían el mismo comportamiento que el número modal de cromosomas. Durante los 3 primeros pasos el número de cromosomas bibraquiales fue de dos, los correspondientes a los cromosomas sexuales X. En los dos últimos el número modal se presentó altamente incrementado, siendo más de tres. En general las alteraciones más comunes de este tipo de tumores fueron células hipodiploides, cromosomas adicionales bibraquiales y monosomía del cromosoma X.

INTRODUCTION

Apart from those in rodents, spontaneous tumours are seen most frequently in dogs, cats and humans. Experimental studies of canine neoplasms, in vitro and in vivo, have increased in number during the last years, mainly to investigate the comparative value of the dog as a model for studies of human tumours (Taylor *et al.,* 1975; Owen *et al.,* 1977; Wolfe *et al.,* 1986; Maiolino *et al.,* 1995). Mast cell tumors are among the most common skin neoplasms in dog (Er and Sutton, 1989; Gross *et al.,* 1992; Rothwell *et al.,* 1987). They tend to occur in middle-aged to elderly dogs (Hottendorf and Nielsen, 1967; Patnaik *et al.* 1984).

The application of the newer techniques of chromosome study to canine neoplasms in the last 7 years has led to significant advances in cancer research. Unfortunately, this has not been matched by similar success in the study of the more common solid tumours. This disparity is attributable mainly to the inherent difficulties of sampling and of making chromosome preparations from solid tissues.

Various forms of cancer have been described in the dog (Oshimura *et al.,* 1973; Mellink *et al.,* 1989; Mayr *et al.,* 1992). Cytogenetic investigations revealed hypodiploid, diploid, hyperdiploid and polyploid chromosome numbers in tumour cells. In many cases additional biarmed chromosomes were observed, which might be the result of centric fusions (Mayr *et al.,* 1990; Mayr *et al.,* 1991b).

The aim of studies of canine tumors in vitro is to increase the understanding of the histogenesis, pathogenesis and chraracterization of the cytogenetic errors involved in the solid neoplasms in the dog and should be of interest for comparative oncology.

Our team has begum working with solid tumors in the dog in order to identify the chromosomic alteration involved in these tumors, as well as the evolution of these alteration throughout the in vitro culture process. The preliminary results are shown in this poster.

MATERIALS AND METHODS

ORIGIN OF THE CULTURES AND ESTABLISHMENT PROCEDURES

A 10-year-old female Braco breed dog was admitted in October 1995 to our veterinary clinic (Small Animal Clinic, Veterinary Faculty, University of Cordoba) with a tumour in the left thigh. After the tumour was surgically removed, postoperative healing of the wound and convalescence were good. Histological examination showed the tumour to be a grade I cutaneous mastocytoma.

Primary explant cell cultures from the tumour were set up by mincing the

solid tissue into small fragments (less than 1 mm). The fragments were transferred into four sterile flasks containing 5 ml TCM-199 medium supplemented with 10 p. cent fetal calf serum and antibiotics (10,000 IU penicillin, 10 mg streptomycin and 25 mg amphotericin/ml). The cultures were incubated in 5 p. cent CO_2 in air at 37°C for three months.

TISSUE CULTURE CHARACTERISTICS

After 24 h culture, a majority of the cells appeared spindle-shaped, and only occasionally were round and ovoid cells observed. Cultures at 5 days clearly showed one morphologic type: a spindle-shaped fibroblast. When primary cultures obtained 75 p. cent or more confluence, cells were detached with the use of 0.25 p. cent trypsin-0.05 p. cent EDTA in Hank's balanced salt solution without magnesium and calcium, transferred to a tube containing 1 ml FCS for inactivating the trypsin and centrifuged at 800 g for 5 min. The cells from two culture flasks were cytogenetically analyzed and other two flasks were resuspended in another two new culture flasks containing 20 ml of TCM-199 medium supplemented with 10 p. cent FCS. Every two days, the medium was changed for new medium and subcultures were generally made about every seven to ten days. When subcultures obtained 75 p. cent or more confluence, the cells of one flask were processed for chromosome analysis, and the cells of another flask were again processed and subcultivated.

In each subculture, the cells showed good growth and almost complete attachment and elongation. This stage of growth was characterized by long, slender, fibroblast-like cells, parallel, and growing in a fusiform. After about five or six weeks, the cultures began showing degenerative changes: bizarre shapes having three to five long protoplasmic extensions and shedding from the glass (passages 4 and 5) resulting in degeneration of the cultures.

CHROMOSOME PREPARATION

The harvesting of cultures was done following the usual protocol and the preparations were air-dried. The metaphases were examined after conventional Giemsa staining and between 25 to 57 metaphases were examined in order to analyse the chromosomes number and the presence of biarmed chromosomes or other chromosome abnormalities.

RESULTS

In the present study, a total of 240 metaphases were analyzed. The frequency distribution of chromosome numbers in the explante and the different passages are shown in **table I** in which the number of chromosomes in different cells was from <73 to >78 chromosomes. In **table II** the frequency distribution of number of the biarmed chromosomes in these different passages are shown.

A total of 40 metaphases were analyzed in the explante cell culture. The modal number was 78 (70 p. cent), 87.5 p. cent of the cells having the normal biarmed number of chromosomes in female dogs.

The number of metaphases analyzed in the 1st passage were 48. Most cells (54 p. cent) showed a modal number of 78 chromosomes, 33 p. cent of the cells showing less than 73 chromosomes. The

Table I. *Frequency distributions of chromosome numbers in different passages.* (Distribución del número de cromosomas en los diferentes pasos).

	Cells observed	Chromosome counts								Cells (p. cent) with modal number (2n=78)
		< 73	73	74	75	76	77	78	> 78	
Explante	40	5	1	-	-	2	3	28	1	70
1st passage	48	16	-	-	2	-	2	26	2	54
2nd passage	40	13	2	1	2	7	-	15	-	37.5
3rd passage	57	17	-	1	5	6	8	19	1	33
4th passage	25	12	1	2	2	2	1	5	-	20
5th passage	30	15	2	3	2	2	1	5	-	17

number of biarmed chromosomes was the same as the explante.

In relation to the 2nd passage, a total of 40 metaphases were analyzed. In this culture, we found a variation for the first time in the former distribution of chromosome. We found a percentage of 32.5 p. cent of cells with < 73 chromosomes and the same percentage (37.5 p. cent) with >78 chromosomes. The number of biarmed chromosomes was the same as the former cultures.

The 3rd passage in which we analyzed 57 metaphases, showed the same distribution in chromosome number and in biarmed chromosomes as the second passage.

In the 4th and 5th passages, 25 and 30 metaphases respectively were analyzed. In these two last passages, the number of 78 chromosomes per cell continued decreasing and those with less than 73 chromosomes were increasing. In relation to the biarmed chromosomes appearing in these pasages, their number was very different than in the former passages. In these cases, the most frequent number was more than three biarmed chromosomes.

Table II. *Frequency distributions of numbers of biarmed chromosomes in different passages.* (Distribución del número de cromosomas bibraquiales en los diferentes pasos).

	Cells observed	Biarmed chromosomes per cell (including X)				
		0	1	2	3	> 3
Explante	40	-	3	35	2	-
1st passage	48	-	2	45	1	-
2nd passage	40	-	7	31	2	-
3rd passage	57	1	6	48	2	-
4th passage	25	-	2	10	3	10
5th passage	30	-	2	8	5	15

DISCUSSION

The mast cell tumour (mastocytoma) is a malignant tumour which is composed of mast cells showing a wide range of granulation both among and within cases. The cells are round, oval, polygonal, or elongated and have oval nuclei. The cytoplasm appears pale red and slightly granular in haemotoxylin and eosin section, and diagnosis is helped by staining with toluidine blue. In less clearly

Figure 1. *Normal morphology of a spindle shaped fibroblast.* (Morfología normal de un fibroblasto en huso).

differentiated mast cell tumours, bizarre cells, possibly multinucleated, may occur (Weiss and Frese, 1974; Gross *et al.*, 1992).

Mast cell tumours are the most common mesenchymal cutaneous neoplasms in the dog and occur very frequently in Boxers, terriers, and Labrador Retrievers (Larsson, 1957; Hottendorf and Nielsen, 1968; Peters, 1969; Bostock *et al.*, 1989; Er and Sutton, 1989; Simoes *et al.*, 1994). They occur also in cats and less frequently in other species. In this study, a mast cell tumour was maintained in culture for three months. The tumour cells were grown vigorously in TCM-199 medium supplemented with 10 p. cent FCS. Serum was necessary for continuous growth-supplying growth factors. Similar results were obtained by Adams *et al.*, (1968) who maintained a canine venereal tumour in continuous culture.

With the primary explante cell culture, an unique morphologic type of cell is always evident: the spindle-shaped fibroblast **(figure 1)**. In the 4th and 5th passage, we observed a morphologic change in the cultured cells, characterized by bizarre shapes with three to five long protoplasmic extensions **(figure 2)**. This cellular change was also observed by Adams *et al.*, (1968) and by Norval *et al.*, (1984) in three canine mammary carcinomas.

To date, numerous reports on cytogenetic studies of canine tumours have been reported (Weber *et al.*, 1965; Miles *et al.*, 1969; Murray *et al.*, 1969; Adams, *et al.*, 1981; Mayr *et al.*, 1995). Cytogenetic investigations have revealed several chromosome abnormalities in dog tumour cells, such as: hypodiploid (Makino, 1974; Cohen, 1985), hyperdiploid (Pool and Wolf, 1974),

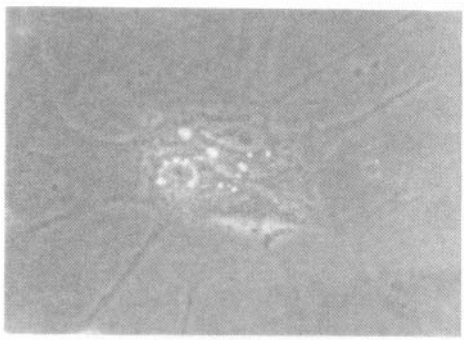

Figure 2. *Degenerative fibroblast showing numerous protoplasmic extensions.* (Fibroblasto degenerado mostrando extensiones protoplasmáticas).

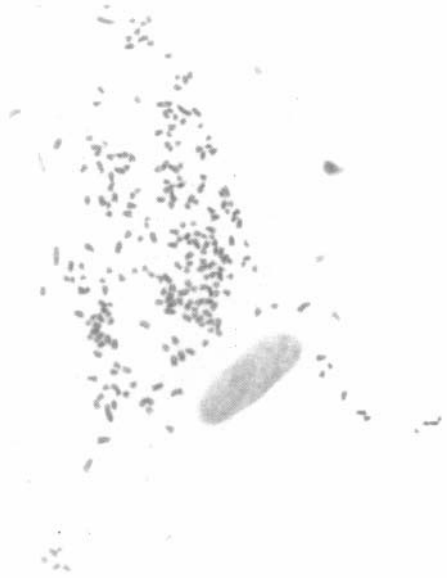

Figure 3. *Metaphase plate showing hyperdiploidy.* (Metafase mostrando hiperdiploidía).

polyploid (Grindem and Buoen, 1986). In many cases additional biarmed chromosomes were observed (Sonoda *et al.*, 1970; Wolf *et al.*, 1972; Mayr *et al.*, 1990; Mayr *et al.*, 1991b). Occasionally, authors mentioned the presence of trisomy, monosomy X, tandem translocation and marker chromosomes (Mellink *et al.*, 1989; Mayr *et al.*, 1991a; Mayr *et al.*, 1993; Mayr *et al.*, 1995).

The present study revealed complex cytogenetic changes in the spindle-cell mastocytoma, a type of tumour for which few data are available. The chromosome number in the different passages ranged from less than 73 chromosomes to more than 78 chromosomes. The modal number in the early three passages was 78 chromosomes, and in the last two passages was less than 73 chromosomes. In the majority of the studied metaphases two biarmed chromosomes were found. However, in the 4th and 5th passages the predominant number of biarmed chromosomes was > 3 chromosomes. Furthermore, important chromosome aberrations similar to those described above were also found in our study, such as: hyperdiploid cells **(figure 3)**, hypodiploid cells, monosomy X, and extra biarmed chromosomes. Hypodiploid cells is a chromosome aberration observed by us in the present study **(figure 4)**. Wolfe *et al.*, (1986) and Mayr *et al.* (1994) also reported the loss of chromosomes in several canine mammary cancers. Rutteman *et al.*, (1988) considered the implication of chromosome loss in canine mammary carcinomas; in their studies hypodiploid cancers seemed to be more frequent than in the human beings (Barlogie *et al.*, 1987; Cornelisse *et al.*, 1987). The

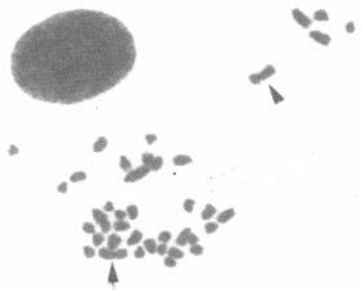

Figure 4. *Metaphase plate showing hypodiploidy with the normal X chromosomes.* (Metafase mostrando hipodiploidía con los cromosomas X normales).

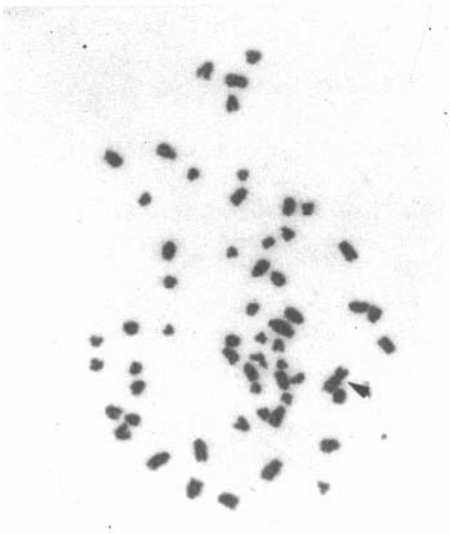

Figure 5. *Metaphase plate showing hypodiploidy involving an X chromosome.* (Metafase mostrando hipodiploidía con un sólo cromosoma X).

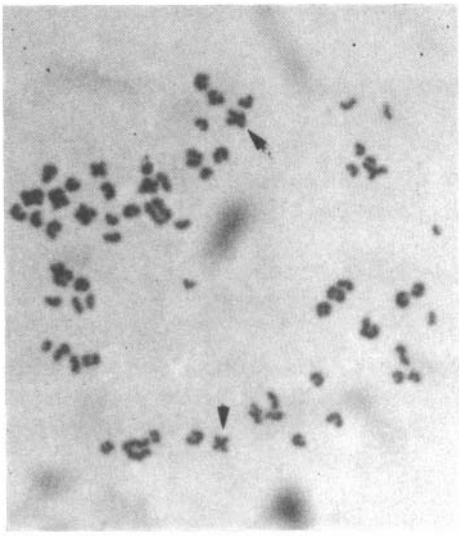

Figure 6. *Metaphase plate showing hypodiploidy and extra biarmed chromosomes.* (Metafase mostrando hipodiploidía y cromosomas bibraquiales).

distribution of genes coding for vital cellular functions over a great number of chromosomes (78 *vs* 46 chromosomes) might enable canine cells to withstand the loss of chromosomes more successfully (Rutteman *et al.,* 1988).

Monosomy X is another chromosome alteration observed in this study **(figure 5)**. This anomalous type of net loss of chromosomal material has been also reported in a few other cases of dog tumours (Grindem and Buoen, 1986; Mellink *et al.,* 1989; Mayr *et al.,* 1991a).

Finally, additional biarmed chromosomes are the most frequent phenomenon in canine neoplasia (Adam and Slaughter, 1970; Makino, 1974; Cohen, 1985; Mayr *et al.,* 1991a) **(figure 6)**, and authors usually conclude that they are the result of centric fusions (Pakes *et al.,* 1965; Barski and Cornefert-Jensen, 1966; Idowu, 1977; Grindem and Buoen, 1986; Mayr *et al.,* 1994). However, Reimann *et al.* (1994) hypothesized that theses biarmed chromosomes are the results of telomeric fusions. In fact, the mechanism leading to these abnormalities are still unknown. The presence of these chromosome alterations is not surprising, because failures of accuracy of chromosome disjunction, leading to aneuploidy, are a general feature of tumour cells (Holliday, 1989; Stone *et al.,* 1991). In our study, it is interesting to note that the number of biarmed chromosomes, in the last two passages, was > 3. This suggests that the predominant cells of these passages were of stemline derivation (Ford and Clarke, 1963).

REFERENCES

Adam, W.A and L.J. Slaughter. 1970. *Pathol. Vet.*, 7: 498-502.

Adams, E.W, L.P. Carter and J.S. Walter. 1968. *Cancer Res.*, 28: 753-757.

Adams, E.W., W.J. Sapp and L.P. Carter. 1981. *Cornell Vet.*, 71: 336-346.

Barlogie, B., M.N. Raber, J. Schumann, T.S. Johnson, A. Drews, D.E. Schwartzendruber, W. Gohde, M. Andreeff and Y. Freireich. 1987. *Cancer Res.*, 43: 3982-3997.

Barski G. and F.R. Cornefert-Jensen. 1966. *J. Natl. Cancer Inst.*, 37: 787-797.

Bostock, D.E., J. Crocker, K. Harris and P. Smith. 1989. *Br. J. Cancer*, 59: 915-918.

Cohen, D. .1985. *Adv. Cancer Res.*, 43: 75-112.

Cornelisse, C.J., R. Caspers, J. Hermans, A.J. Molenaar and C.H.J. Velde. 1987. *Cytometry*, 8: 225-234.

Er, J.C. and R.H. Sutton. 1989. *Aust. Vet. J.*, 66: 225-227.

Ford, C.E. and C.M. Clarke. 1963. *Canad. Cancer Conf.*, 5: 129.

Grindem, C.B. and L.C. Buoen. 1986. *J. Comp. Pathol.*, 96: 623-635.

Gross, T.L., P.J. Ihrke and E.J. Walder. 1992. In: Veterinary Dermatology, de. Reinhardt RW, 1st de., pp. 465-473.

Holliday, R. 1989. *Trends in Genetics*, 5: 42-45.

Hottendorf, G.H. and S.W. Nielsen. 1968. *Pathol. Vet.*, 5: 102-121.

Idowu, L. 1977. *Res. Vet. Sci.*, 22: 271-273.

Larsson, B. 1957. *Veterinarmed*, 9:241.

Makino, S. 1974. In: German, J. (de), Chromosomes and Cancer, New York, Wiley & Sons, pp 335-372.

Maiolino, P, B. Restucci and G. De Vico. 1995. *J. Vet. Med. A.*, 42: 339-343.

Mayr, B., W. Swidersky, W. Schleger and M. Reifinger. 1990. *Br. Vet. J.*, 146: 260-263.

Mayr, B., H. Gilli, W. Schleger, M. Reifinger and H. Burtscher. 1991a. *J. Vet. Med. A.*, 38: 141-147.

Mayr, B., H. Kofler, W. Schleger, G. Loupal and H. Burtscher 1991b. *Res. Vet. Sci.*, 50: 298-300.

Mayr, B., U. Eschborn, W. Schleger, G. Loupal and H. Burtscher. 1992. *J. Comp. Path.*, 106: 319-322.

Mayr, B., U. Eschborn, G. Loupal and W. Schleger. 1993. *Vet. Pathol.*, 30: 311-313.

Mayr, B., M. Reifinger, H. Weissenbock, W. Schleger and E. Eisenmenger. 1994. *Res. Vet. Sci.*, 57: 88-95.

Mayr, B., M. Scheller, M. Reifingerand G. Loupal. 1995. *Br. Vet. J.*, 151: 433-441.

Mellink. C.H.M., A.A. Bosma and G.R. Rutteman. 1989. *Anticancer Res.*, 9: 1241-1244.

Miles, C.P., G. Moldavannu, D.G. Miller and A. Moore. 1970. *Am. J. Vet. Res.*, 31: 783-790.

Murray, M., Z.H. James and W.B. Martin. 1969. *Res. Vet. Sci.*, 10: 565-568.

Norvall, M., J. Maingay and R.W. Else. 1984. *Eur. J. Cancer Clin. Oncol.*, 20: 1489-1500.

Oshimura, M., M. Sasaki and S. Makino. 1973. *J. Natl. Cancer Inst.*, 51: 1197-1203.

Owen, L.N., D.R. Morgan, D.E. Bostock and R.J. Flemans. 1977. *European J. Cancer*, 13: 1445-1449.

Pakes, S.P., R.A. Griesemer and L. Kasza. 1965. *Am. J. Vet. Res.*, 26: 837-843.

Patnaik, A.K., W.J. Ehler and E.G. MacEwen, 1984. *Vet. Pathol.*, 21: 469-474.

Peters, J.E. 1969. *J. Natl. Cancer Inst.*, 42: 435-443.

Pool, R.R. and H.G. Wolf. 1974. *Cancer*, 34: 771-779.

Reimann, N., P. Rogalla, B. Kazmierczak, U. Bonk, Y. Nolte, T. Grzonka T. Bartnitzke and J. Bullerdiek. 1994. *Cytogenet. Cell Genet.*, 67: 81-85.

Rothwell, T.L.W., C.R. Howlett; D.J. Middleton D.A. Griffiths and B.C. Duff. 1987. *Aust. Vet. J.*, 64: 161-164.

Rowley, J.D.. 1974. *J. Natl. Cancer Inst.*, 52: 315-320.

Rutteman, G.R., C.J. Cornelisse, N.J. Dukshoorn, J. Poortman and W. Misdorp. 1988. *Cancer Res.*, 48: 3411-3417.

Simoes, J.P.C., P. Schoning, M. Butine. 1994. *Vet. Pathol.*, 31: 637-647.

Sonoda, M., M. Niiyama and M. Mori. 1970. *Jap. J. Vet. Res.*, 18: 145-151.

Stone, D.M., P.B. Jacky and D.J. Prieur. 1991. *Cancer Genet. Cytogenet.*, 53: 105-112.

Taylor, N., M. Shifrine, H.G. Wolf and A. Trommershausen-Smith. 1975. *Transplant Proc.*, 7: 485-493.

Weber, W.T., C. Nowell and W.C.D. Hare. 1965. *J. Natl. Cancer Inst.*, 35: 537-547.

Weiss, E. and K. Frese. 1974. *Bull. Wld. Hlth. Org.*, 50: 79-100.

Wolf, H.G., N.J. Taylor, S.J. Calligan and K. Shimoto. 1972. In: Annual Report, Radiobiology laboratory, UCD 472.119, Davis, University of California, pp. 91-94.

Wolfe, L.G., B.B. Smith, M.A. Toivio-Kinnucan, E.A. Sartin, R.P. Kwapien, R.A. Henderson and S. Barnes. 1986. *J. Natl. Cancer. Inst.*, 77: 783-792.