

# CLONAL EVOLUTION OF A MOUSE METASTATIC HEMANGIOSARCOMA

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**Key words:** tumor, bone marrow, chromosomes, clone

**Abstract:** In a mouse metastatic hemangiosarcoma chromosome changes were assessed by chromosomal preparations made from tumors and bone marrow. G-banded chromosomes revealed a consistent abnormal clone: 40,XX,ace which occurred in all probes. This partial clonal trisomy was associated with sidelines distinguished by different secondary chromosome anomalies added to primary aberration. The implications of chromosomes X, 14, 17 and 18 in numerical and structural anomalies was striking enough.

## INTRODUCTION

In man the incidence of hemangiosarcoma is higher than in other mammals. Of those species, the dog and the horse are more frequently affected (Johnson 1987; Scott and Gourreau 1996), and the occurrence of spontaneous hemangiosarcomas is sporadic in laboratory animals like mouse (Chandra and Frith 1992).

Although vascular tumors are histopathologically better studied both in man and other mammals, including mouse (Booth and Sundberg 1995), the cytogenetic profile of hemangiosarcoma was poorly investigated.

In man, when cytogenetic analyses were performed, the method used was short-term tumoral cell culturing (Mandahl et al. 1990; Schuborg et al. 1998). Thus the chromosomal changes could not be evaluated as random or nonrandom.

## THE AIM OF INVESTIGATIONS

The cytogenetic study of a mouse metastatic hemangiosarcoma was achieved with two objectives:

1. to obtain more information about characteristic chromosome changes;
2. to assess an eventual clonal evolution.

## MATERIAL AND METHODS

In the Swiss mouse colony of the Faculty of Veterinary Medicine Iasi, Laboratory of Cytogenetics, in a female, a subcutaneous tumor-like mass of soft consistency appeared spontaneously on the right side of the thorax just back to the scapula. The size of the tumor was 1.2 x 0.5 x 0.3 cm. Clinical examination evidenced also the presence of another subcutaneous tumoral mass, smaller (0.6 x 0.3 x 0.2 cm), with the same soft consistency, localized in the left inguinal region.

The individual was isolated and after 4 days her condition became critical, and therefore was killed.

Forty five minutes before killing, an 0.04% colchicine solution was injected in 2 successive doses, and sites of administration: 0.5 ml directly into the principal larger tumor, and 0.3 ml, intraperitoneally, after 30 minutes from the first inoculation.

Chromosome preparations were made from both tumors and bone marrow by classical method (Raicu et al. 1983).

The G-banding was obtained by ASG technique (Sumner et al. 1971).

## RESULTS AND DISCUSSION

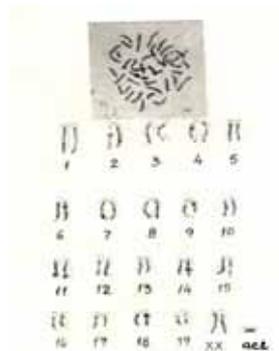
Gross observations evidenced that the two tumor-like masses consisted of many blood clottings. This aspect together with the result of histopathologic examination (numerous sinusoidal blood vessels, larger blood filled spaces) indicated that the two tumors are hemangiosarcomas, the first, larger, being the primary tumor and the second one, with smaller dimensions, being the secondary tumor as a consequence of the metastasis.

Regarding the chromosome examination in primary tumor, out of 42 metaphases only 28 were analysable. Cytogenetic results of these metaphases are presented in Table1.

**Table 1 - The cytogenetic profile of the primary mouse tumor**

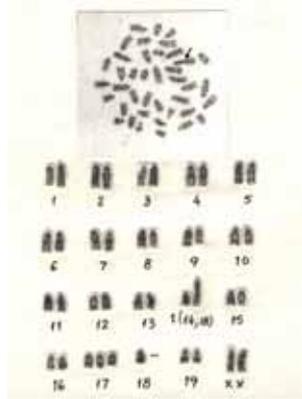
Condition	Chromosome findings	No. of cells	%
Normal clone	40,XX	20	71.3
Abnormal clone	40,XX,ace	3	10.7
Abnormal subclone	39,X,ace	1	3.6
Structural aberrations	40,XX,del(XF)	1	3.6
	40,XX,r(17)	1	3.6
Numerical aberrations	39,XX,+1,-16	1	3.6
Numerical + structural aberrations	41,XX,+17,rob(14;18)	1	3.6

The karyotype was normal: 40,XX in 20 cells, i.e. 71.3% of analysed cells. Three cells (10.7%) showed the similar anomaly: the presence of an acentric fragment. This observation was interpreted as a clonal partial trisomy (Fig. 1).



**Fig. 1 - Acentric fragment as the marker of the clonal partial trisomy in the mouse primary tumor**

One cell (3.6%) presented an X monosomy associated to clonal acentric fragment. The other 4 cells (14.4%) were all single-cell with numerical and structural abnormalities. As structural aberrations there were markers: the deletion of the F region of an X gonosome and a ring chromosome 17. As numerical aberrations were found: a complex anomaly (a trisomy 1 added to a nullisomy 16) and also an association of a numerical aberration with a structural one (a trisomy 17 + a rob translocation 14;18 (Fig. 2).



**Fig. 2 - Association of a trisomy 17 with a 14;18 rob translocation in the mouse primary tumor**

In secondary tumor chromosome preparations 9 karyotypes were studied and revealed normal 40,XX cells (44.3%), the presence of the acentric fragment marker in 22.1% of cells and the remaining cells evidenced structural aberrations and numerical anomalies (monosomy 8).

The results of bone marrow chromosome examination are presented in Table 2.

**Table 2 - The cytogenetic profile of the mouse bone marrow**

Condition	Chromosome findings	No. of cells	%
Normal clone	40,XX	68	88.3
Abnormal clone	40,XX,ace	2	2.6
Abnormal subclones	41,XX,del(XF),+16,ace	1	1.3
Structural aberrations	39,XX,+7,-18,ace	1	1.3
Structural aberrations	40,XX,del(XF)	1	1.3
	40,XX,rob(14;18)	1	1.3
Numerical aberrations	39,XX,-8	1	1.3
	40,XX,+1,-14	1	1.3
	41,XX,+19	1	1.3

In 68 cells (88.3%), the karyotype was normal: 40,XX. Two cells (2.6%) showed the clonal chromosome complement: 40,XX,ace, observed also in some cells of the primary tumor and the secondary tumor. Another two cells (2.6%), besides the clonal acentric marker, contained additional aberrations. Thus a cell was characterized by an association of an X chromosome terminal deletion with atrisomy 16, and the other cell demonstrated trisomy of 7, and monosomy of 18.

There were also present structural aberrations in 2 cells (2.6%) represented by a terminal deletion of an X chromosome and a rob translocation 14;18 which were observed in the primary tumor too. The numerical aberrations, seemingly random, were present in 3 cells (3.9%). They were a monosomy 8, a trisomy 19 and a trisomy 1 added to a monosomy 14.

It was not possible to establish the origin of the acentric fragment occurring in more cells examined from the primary and secondary tumors, and from bone marrow.

According to the modern theory of cancer pathogenesis, the neoplasm is the result of a somatic cell mutation, followed by a clonal proliferation of its descendants with characteristic chromosomal anomalies. A clonal population is defined by the detection of at least three cells with the same chromosome loss or two sharing the same chromosome gain or structural abnormality.

On the basis of these principles, the cytogenetic analysis presented here on the mouse metastatic hemangiosarcoma shows its monoclonal origin. The primary aberration is presented by an acentric fragment evidenced in 2-3 cells from each examined specimen. Thus this unidentified partial trisomy characterizes the stemline of this hemangiosarcoma. Similar anomaly was described in a hemangiopericytoma in dog (Mayr et al. 1994).

The clonal evolution of this studied hemangiosarcoma is emphasized by the presence of related subclones. They arose through the acquisition of new chromosome aberrations: 39,XX,ace (primary tumor); 40,XX,rcp(3;9),ace (secondary tumor); 41,XX,del(XF),+16,ace and 39,XX,+7-18,ace (bone marrow).

Gradually during the tumor progression, other two clones emerged with the structural anomalies like terminal deletion of an X chromosome (40,XX,del(XF)), and the rob translocation 14;18 in the primary tumor, both present also in the bone marrow.

Other structural aberrations (rcp translocation 10;18 and ring chromosome 17) and single (trisomy 19) or complex numerical aberrations (monosomies added to trisomies) occurred only in a single cell. Therefore these modifications are interpreted as random cytogenetic changes, representing "cytogenetic noise".

The advanced stage of tumor as considered by the critical condition of the animal justifies this increased level of cytogenetic complexity with clonal changes and others representing genetic instability.

As it could be observed from the tables, in the present metastatic hemangiosarcoma, the numerical chromosome anomalies as aneuploidies prevailed over the structural chromosome aberrations. Among numerical anomalies dominated those with partial and total gain, their importance being evident by the fact that they have been demonstrated to be clonal and subclonal.

Although the cytogenetic picture obtained in this hemangiosarcoma by the direct analysis of the primary tumor and its metastases is probably incomplete, nevertheless it is clear that the level of cytogenetic complexity increased as the tumor progressed to the last stage.

## CONCLUSIONS

A constant, unidentified partial trisomy (40,XX,ace) characterizes the primary clone remarked in the primary tumor, the secondary tumor and the bone marrow.

This clonal primary line is accompanied by the sidelines characterized by different secondary chromosome anomalies which were singles or added to the primary aberration.

The clonal, nonrandom primary and secondary aberrations have been associated with multiple nonclonal, random chromosome changes especially in bone marrow as a result of genomic instability of final stage of metastatic hemangiosarcoma development.

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