Chromosomal Mapping of the Metastasis Associated *MTS1* Gene by *in situ* Hybridization

M. S. Lakshmi, C. Parker and G. V. Sherbet¹

Cancer Research Unit, The Medical School, University of Newcastle upon Tyne,
Newcastle upon Tyne, NE2 4HH, UK

Accepted January 23, 1992

Several genes have been found to be differentially expressed in malignant tumours and their benign counterparts. Among these is the *MTS1* gene reported to be over expressed in high metastasis murine tumours and human tumours grown as xenografts (Ebraldze *et al.* 1989, 1990). We have now confirmed this empirical association between *MTS1* expression and tumour malignancy with the finding that modulators of metastatic behaviour alter the expression of the *MTS1* gene in metastatic variants of the B16 murine melanoma (Parker *et al.* 1991). In the light of the increasing evidence of a role for *MTS1* in the secondary spread of cancer, we have mapped the gene by chromosomal *in situ* hybridization to chromosome 7q22.

Materials and methods

Lymphocyte cultures and preparation of metaphase spreads

Human lymphocytes from short term cultures were used in this study. The cells were treated with colcemid (0.2 µg/ml) for 2 hr at 37°C before harvesting, pelleted by centrifugation at 1700 r.p.m. for 3 min., resuspended in 0.075 M KCl and incubated at 37°C for 8 mins. The cells were then softly pelleted and resuspended in about 150 µl of Ca²⁺–Mg²⁺ free Dullbecco A solution, and fixed by trickling 10 ml of freshly prepared fixative containing methanol (3 vols.) and glacial acetic acid (1 vol.). Treatment with the fixative was repeated thrice. The lymphocytes were pelleted and resuspended in 0.5–1.0 ml of the fixative. The cells were dropped on to clean grease-free slides and allowed to dry. A few drops of methanol were trickled over the cells and the slides were dried at 37°C in a humidified chamber for 24 hr and stored for 2 weeks. The metaphase chromosomes were stained with Wright's stain as described by Benn and Perle (1986).

*Labelling of the *MTS1* probe*

The murine 18A2 (Jackson-Grusby *et al.* 1988)/*MTS1* RNA probe was labelled with 5, 6 ³H-UTP using the paired promoter T7 system (Amersham). The separation of the incorporated label from the unincorporated was carried out using a Sephadex G-50 NICK column (Pharmacia). The total amount of label incorporated was 2.1 × 10⁷ cpm in 600 µl 1 × TE buffer, and 1.3 × 10⁶ counts were added to each slide.

In situ hybridization and autoradiography

Chromosomal hybridization using the *MTS1* RNA probe was carried out as described by Pardue (1985). The hybridized sequences were visualised by dip emulsion autoradiography using high resolution emulsion LM1 (Amersham). The slides coated with the emulsion were exposed in a light-proof box for 16 days at 4°C and then developed with Kodak LX24 X-ray developer for 8 min at 4°C, fixed in Kodak FX40 X-ray liquid fixer for 8 min at 4°C.

¹ Correspondence to: Dr G. V. Sherbet

The developed slides were stained with Wright's stain in 0.06 M phosphate buffer at pH 6–8, for 10 min at room temperature, destained and restained in Wright's stain. The slides were air dried overnight, taken through xylene for 5 min and mounted in Depex mountant with 'O' thickness cover glasses.

Chromosome typing by Magiscan MD

The chromosomes, with grains touching or on the chromatids, were typed using the Joyce Loeb Image Analysis system Magiscan MD with the aid of the chromosome software. The typing was confirmed by using the chromosome profile option and checking their staining pattern.

Result and discussion

The metaphase spreads that were hybridized with the *MTS1* probe showed on average 2.3 silver grains on or touching the chromosomes per spread. Fig. 1 shows the distribution of

Fig. 1. Location of silver grains from hybridization of ^3H -labelled *MTS1* probe based on 54 metaphase spreads.

123 silver grains found in 54 metaphase spreads. Four chromosomes, viz. 7, 10, 13, and 15 showed more silver grains associated with them than can be expected on the basis of percentage of haploid genomic DNA in the chromosome (Table 1). The silver grain distribution was 15.4% on chromosome 7, 10.5% on chromosome 10, 13.8% on chromosome 13, and 11.4% on chromosome 15. Of these four chromosomes, the localisation of the silver grains with specific bands of chromosomes 10 and 15 was regarded as random distribution. Only one or two grains were associated with individual bands. This was statistically not significant since five or more grains per band were required to reach statistical significance at $p < 0.05$. In contrast, the distribution of the grains on chromosome 7 was not random and 37% of the grains on this chromosome were localised to q22 (Fig. 2). This was statistically highly significant at $p < 0.008$ in Fisher's exact test.

On chromosome 13, 23.5% of the grains were localised to q22 and 17.6% to q21.3 (Fig. 3). On this chromosome the presence of 5 (29%) or more grains per band was required to reach statistical significance in Fisher's exact test at $p=0.044$. One may conclude therefore that

Table 1. Silver grain distribution on human chromosomes annealed with [^3H]-MTS1

Chromosome No.	Observed Grains	Expected* Grains	χ^2
1	2	10.4	
2	2	10.3	
3	1	8.61	
4	4	8.01	
5	1	8.01	
6	11	7.08	
7	19	7.08	20.05
8	2	6.42	
9	3	5.73	
10	13	5.73	9.22
11	2	5.75	
12	0	5.75	
13	17	4.4	36.08
14	4	4.4	
15	14	4.4	20.9
16	1	3.8	
17	1	3.6	
18	0	3.4	
19	0	2.8	
20	0	2.8	
21	3	2	
22	2	2	
X	8	6.4	

* Expected grains calculated on the basis of the DNA content of chromosomes (Mayall *et al.* 1975).

Fig. 2. Distribution of silver grains on chromosome 7 based on 54 cells.

Fig. 3. Distribution of silver grains on chromosome 13 based on 54 cells.

although hybridization signals were detected in the 13q21-q22 region, hybridization of the probe to neither q21 nor q22 was statistically significant ($p=0.1$). Comparing the pattern and statistical significance of the localization of the silver grains on chromosomes 7 and 13, we suggest that 7q22 is the major site of hybridization and the locus of the *MTS1* gene. Collard *et al.* (1987) have presented evidence based on cell fusion studies for the existence of genes controlling invasion and metastasis on chromosome 7. Recently Bertrand *et al.* (1990) have reported that in human melanoma cell lines the occurrence of multiple copies of chromosome 7 is associated with metastatic ability. Viewed in the light of these report our assignment of the metastasis associated *MTS1* gene to 7q22 would seem significant.

Summary

The *MTS1* gene is a metastasis associated gene. *In situ* hybridization of human lymphocyte metaphase chromosomes with 18A2/*MTS1* RNA probe has mapped the gene to chromosome 7q22.

Key words: *MTS1* gene, cancer metastasis, in situ hybridisation.

Acknowledgement

This work was supported by the North of England Cancer Research Campaign and the Tom Berry Memorial Fund.

References

- Benn, P. A. and Perle, M. A. 1986. Chromosome staining and banding techniques. In: Human Cytogenetics: A Practical Approach, eds. Rooney DE, Czepulkowski BH. IRL Press, Oxford, pp. 57–84.
- Bertrand, S., Bailly M., Nguyen M. J. and Dore J. F. 1990. Cytogenetic characterization of human melanoma metastatic variants in immunosuppressed new born rat: Role of chromosomes 7 and double minutes. Clin. Exptl. Metastasis 8: Suppl. 1: 35.
- Collard, J. G., van de Poll, M., Scheffer, A., Roos, E., Hopman, A. H. M., Geurts van Kessel, A. H. M. and Van Dongen, J. J. M. 1987. Location of genes involved in invasion and metastasis on human chromosome 7. Cancer Research, 47: 6666–6670.
- Ebralidze, A., Tulchinsky, E., Grigorian, M., Afanasyeva, A., Senin, V., Revazova, E. and Lukanidin E. 1989. Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to Ca^{2+} binding protein family. Genes Develop. 3: 1086–1093.
- , Florence, V. A., Lukanidin, E. and Fostad, O. 1990. The murine *MTS1* gene is highly expressed in metastatic but not in non-metastatic human tumour lines. Clin. Exptl. Metastasis 8: Suppl. 1: 35.
- Jackson-Grusby, L., Swiergiel, J. and Linzer, D. I. H. 1988. A growth related mRNA in cultured mouse cells encodes a placental calcium binding protein. Nucl. Acid Res. 15: 6677–6690.
- Mayall, B. H., Carrano, A. V., Moore II, D. H., Ashorth, L. K., Beannett, D. E., Bogart, E., Littlepage, J. L., Minkler, J. L., Piluso, D. L. and Mendelsohn, M. L. 1975. Cytophotometric analysis of human chromosomes. In: Automation of Cytogenetics, (ed) Mendelsohn BM, U. S. Energy Research and Development Administration, Washington, D. C., pp. 135–144.
- Pardue, M. L. 1985. In situ hybridization. In: Nucleic Acid Hybridization—A Practical Approach, eds. Hames BD, Higgins SJ, IRL Press, Oxford, pp. 179–202.
- Parker, C., Whittaker, P. A., Weeks, R. J., Thody, A. J. and Sherbet, G. V., 1991. Modulators of metastatic behaviour alter the expression of metastasis-associated genes *MTS1* and *NM23* in metastatic variants of the B16 murine melanoma. Clin. Biotechnology, 3: 217–222.