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Review paper

Double minute chromosomes and homogeneously staining regions in tumors taken directly from patients versus in human tumor cell lines

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There is increasing evidence that copies of amplified oncogenes or drug-resistant genes located on extrachromosomal DNA (e.g. double minutes and/or episomes) can be eliminated from mammalian tumor cell lines by treatment of the cells with low concentrations of hydroxyurea. However, amplified oncogenes or drug-resistant genes located in an intrachromosomal site (such as in a homogeneously staining region (HSR)) cannot be eliminated from the cells. A question which arises is do primary human tumors have extrachromosomal DNA present often enough to make elimination of that extrachromosomal DNA a potentially important therapeutic strategy? To address that question we have reviewed published cytogenetic analyses of 200 tumors taken directly from patients to determine the percentage of primary human tumors which have amplified genes present on extrachromosomal DNA (present in the form of double minutes [DMs]) vs the percentage of tumors which have amplified genes located on an intrachromosomal site (in the form of HSRs). Of the 200 primary human tumors reviewed, 91% contained DMs only, 6.5% contained HSRs, and 2.5% contained both. Of interest, in a parallel review of 109 cell lines with cytogenetic and/or molecular evidence of gene amplification, 60.6% contained DMs, 26.6% contained HSRs, and 12.8% contained both. These data indicate that DMs are the predominant cytogenetic marker for gene amplification in patients, but are present less frequently in established cell lines. These findings indicate that ongoing efforts to eliminate amplified drug-resistant genes or oncogenes contained on DMs (or precursors of DMs) from tumor cells may be relevant for *in vivo* situations.

Key words: Double minute chromosomes, gene amplification.

Introduction

Double minute chromosomes (DMs) and homogeneously staining regions (HSRs) are cytogenetic manifestations of gene amplification. Amplified drug-resistant genes and amplified oncogenes have been localized to these structures. This localization makes DMs and HSRs targets of interest for modulation of drug resistance or tumor progression.

Overview of DMs

The occurrence of DMs in a malignant cell line was first described by Spriggs *et al.* in 1962.[1] DMs lack centromeres and consequently have unequal separation at cell division.[2] They tend to cluster at the ends of chromosomes, and are drawn with them during metaphase.[3] DMs tend to have a wide variation in size, even in a specific cell line.[4,5] There is also great variation in the number of DMs per cell. These variations, especially in size, make the DMs more difficult to find.

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In recent months there has been a renewed interest in these structures. There is new molecular evidence that DMs can emerge from small (submicroscopic) circular DNA (episomes) which multimerize over time to become visible as DMs.[6–11] That mechanism of DM formation could help explain the variable size and number of double minutes.

Overview of HSRs

HSRs are areas within the chromosomes which stain similarly throughout their length, rather than demonstrating the typical alternating bands.[12] Within a cell line, HSRs tend to occur most commonly on specific chromosomes.[13,14] The chromosome which is involved varies between cell lines.

Loss of amplified genes

In 1983, Snapka and Varshavsky reported that the rate of loss of unstably amplified genes for dihydrofolate reductase (DHFR) located on DMs in methotrexate-resistant mouse cells could be greatly accelerated by growing the cells in 50 μM (a non-lethal concentration) hydroxyurea.[15] They were not able to determine a mechanism for that accelerated loss.

In the past few months there have been studies indicating that hydroxyurea at concentrations of 50–200 μM can accelerate loss of amplified CAD genes from Chinese hamster ovary cells as well as amplified *mdrl* or *dhfr* genes from human tumor cell lines.[16,17] In addition, hydroxyurea has also been found to cause loss of copies of amplified c-myc and N-myc oncogenes from a variety of human tumor cell lines.[18] Of particular importance is that the hydroxyurea-induced accelerated loss of drug-resistant genes has resulted in a cell population more sensitive to the selective agent. The hydroxyurea-induced loss of oncogene copy number has resulted in a very significant decrease in the ability of the cells to clone in soft agar.[18]

In the above studies, important control cell lines having amplified copies of drug-resistant genes or oncogenes located on an intrachromosomal site (HSR) were also examined. Hydroxyurea caused no accelerated loss or elimination of drug-resistant genes or oncogenes when these amplified genes were intrachromosomally located.

A question which arises is do primary human tumors have extrachromosomal DNA present often enough to make elimination of that extrachromosomal DNA a potentially important therapeutic strategy? To address that question we have reviewed the published cytogenetic analyses of 200 tumors taken directly from patients to determine the percentage of primary human tumors which have extrachromosomal evidence of gene amplification (in the form of DMs) vs the percentage of those with intrachromosomal evidence of gene amplification (in the form of HSRs). The same analyses have also been performed on 109 human tumor cell lines.

There have already been several excellent reviews on DMs and HSRs. Barker has described the structure of DMs,[19] Biedler has described the possible role of DMs and HSRs in neuroblastoma,[20] and Cowell has described both structures in the context of gene amplification.[21] However, none of these reports have focused on the presence of DMs or HSRs in tumors taken directly from patients without intervening tissue culture.

Materials and methods

The data for this review were obtained by using the key words, double minutes, and homogeneous, in a miniMEDLINE search of titles. Once a pertinent article was found, further references were often found in the bibliography. In addition, the tables of content for the journals, *Cancer Research* and *Cancer Genetics and Cytogenetics* were scanned back to 1981. An attempt was made to find additional cases by reviewing articles which discussed tumor karyotypes. Only articles which described human specimens were included. If the Methods section of a reference suggested that the specimen was kept longer than 25 h, it was considered to be a culture.

It is understood that the approach used is not complete. Reliance on bibliographies to find further references may skew the results, by over-representing specific research groups. Also, DMs and HSRs are often not mentioned in the title, but are only mentioned in the text of the article, usually within a karyotype. Neither abnormality is listed separately in the *Index Medicus*. It is difficult to know if the absence of DMs in a karyotype reflects an absence of the structures, or an absence of an attempt to find them. The reporting of HSRs is complicated by the lack of uniformly used nomenclature and staining methods. HSRs may be noted in a karyotype only as marker chromosomes, and their significance lost.

Results

DMs and HSRs in specimens taken directly from patients

Table 1 lists 75 leukemia or myelodysplastic syndrome specimens which had DMs or HSRs on direct examination, with no intervening cultures. Of the cases in Table 1, 74 describe the presence of only DMs. The percentage of cells in each specimen with DMs ranged from 7 to 100% (median 61%). In only one case was an HSR found. This tumor also had DMs (Figure 1A). Most of the patients in Table 1 had myeloid leukemias. Interestingly, three of the myelodysplastic patients with DMs progressed to acute myelogenous leukemia. Nine patients in Table 1 had a previous malignancy, most often Hodgkin's disease or breast cancer. Also, eight patients had received chemotherapy or radiation therapy prior to the karyotyping of their tumor.

As mentioned above, because this is a retrospective review of the literature there are inherent problems such as uncertainties in reporting methods. Perhaps care was not taken to look for DMs and HSRs in the same specimens or perhaps appropriate techniques such as trypsin Giemsa staining were not employed. Therefore, an attempt was made to determine which of the above reports definitely would have described DMs and/or HSRs, if present. Reports which included both abnormalities (i.e. which specifically mentioned both DMs and HSRs), or reports of recent complete karyotypes (with trypsin Giemsa staining) were included in this subset analysis. Using these criteria for the leukemias, there were 29 of these totally evaluable specimens (papers which would have reported DMs or HSRs if present). Twenty-eight had DMs and one specimen had DMs plus an HSR (Figure 1B). We report both total specimens examined and totally evaluable cases to help minimize reporting bias in this retrospective review.

Reports describing DMs and HSRs in solid

Table 1. DMs and HSRs observed in the direct examination of leukemias and myelodysplastic syndromes

Malignancy	Specimen	Age/Sex	Previous malignancy/ treatment	DMs (% cells)	HSRs	Reference
Refractory Anemia	BM	50F	None	+(68)	ND	22
AML (M2)	BM	68F	None	+(33)	No	23
AML (M1)	BM	61F	None	+(7)	No	
Preleukemia	BM	52F	None	+(43)	No	24
AML (M2)		(same pt)	None	+(50)	No	
AML (M2)	BM	60F	None	+(74)	No	25
AML (M2)	BM	70F	ND	+	No	26
AML (M6)	BM	77F	Breast ca, RT, CT	+(50)	ND	27
AML (M2)	BM	66F	Bladder ca, RT	+(100)	ND	
AML (M2)	BM	50M	None	+(26)	ND	28
AML (M4)	BM	53M	None	+(90)	ND	
Preleukemia	BM	77F	Uterine ca, RT, rectal ca	+(69)	ND	
AML (M4)	BM	(same pt)		+(80)	ND	
AML (M4)	BM	65M	None	+(42)	ND	
AML (M4)	BM	70F	Breast ca	+(73)	ND	
AML (M4)	BM	64M	None	+(68)	ND	
Preleukemia	BM	41M	None	+(98)	ND	
AML (M4)	BM	77F	None	+(62)	ND	
AML (M4)	BM	59F	None	+(45)	ND	
AML (M4)	BM	74F	None	+(33)	No	29
AML (M4)	BM	64F	None	+(55)	No	
AML (M4)	BM	78F	Colon ca	+(100)	No	
AML (M6)	BM	77M	None	+(12)	No	
AML (M4)	BM	74F	Breast ca	+(62)	No	
AML (M6)	BM	70F	None	+(61)	No	30
AML (M5)	BM	17M	None	+(50)	No	31

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Table 1. *(continued)*

Malignancy	Specimen	Age/Sex	Previous malignancy/ treatment	DMs (% cells)	HSRs	Reference
AML (M1)	BM	70M	Radiation exposure	+(100)	No	
AML (M1)	BM	51M	None	+(100)	No	32
AML	BM	49M	ND	+	No	
AML	BM	21M	ND	+	No	33
AML (M4)	BM	74M	None	+(14)	No	34
AML	BM	(10 pt)	ND	+	ND	35
Smoldering leukemia		(1 pt)	ND	+	ND	
CML	BM	(6 pt)	ND	+	ND	
Sideroblastic		(4 pt)	ND	+	ND	
Acute leukemia	BM	(3 pt)	Hodgkins	+	ND	
AML (M6)	BM	(2 pt)	ND	+	ND	
Aplastic anemia		(1 pt)	ND	+	ND	
Lymphosarcoma cell Leukemia		(1 pt)	ND	+	ND	
CML, blast crisis	BM	33F	CT	+(81)	No	36
AML	BM	78F	None	+(50)	No	37
AML	BM	66F	RT, CT	+(17)	No	38
AML (M6)	BM	68F	Hodgkins, RT	+	No	39
Preleukemia	BM	51F	PDL, RT	+	No	
Blast phase		(same pt)		+	No	
AML (M6)	BM	72F	ND	+	ND	40
AML	BM	57F	ND	+	ND	
AML	BM	51F	ND	+	ND	
AML	BM	59M	ND	+	ND	
Erythroleukemia	BM	59M	ND	+	ND	
Sideroblastic Anemia	BM	38M	ND	+	ND	41
CMML	BM	60M	RT, CT	+	+	42
	BM	84F	ND	+	No	
	BM	60M	ND	+	No	
	BM	60M	ND	+	No	
Mixed leukemia	BM	65F	ND	+	ND	43
Myeloid metaplasia		F	None	+	No	44

AML, acute myelogenous leukemia; BM, bone marrow; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; CT, chemotherapy; RT, radiotherapy; ND, not determined or stated in original paper; pt, patient; PDL, poorly differentiated lymphoma.

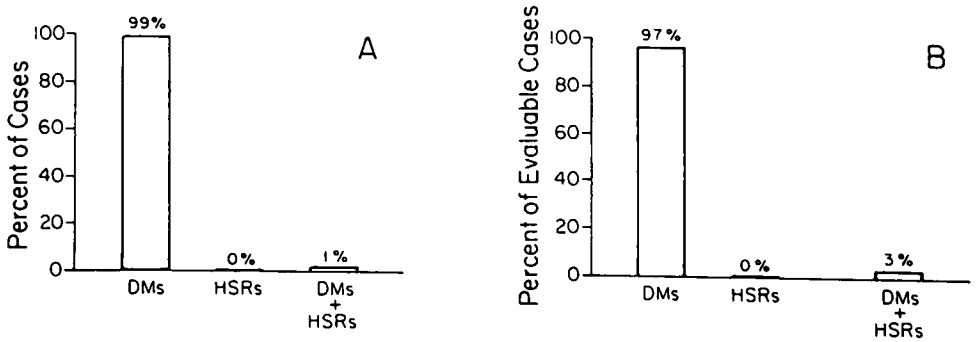

Figure 1. (A) Per cent of leukemia and myelodysplastic syndrome cases examined directly from patients with DMs, HSRs, or both (total cases reviewed). Of 75 cases reviewed, 74 (99%) had DMs only, none had HSRs only, and 1 (1%) had DMs and an HSR reported. (B) Per cent of totally evaluable (see text) leukemia and myelodysplastic syndrome cases examined directly from patients with DMs, HSRs, or both. Of 29 evaluable cases 28 (97%) had DMs only, none had HSRs only, and 1 (3%) had DMs and an HSR reported.

Table 2. DMs and HSRs observed in the direct examination of solid tumors

Malignancy	Specimen	Age/Sex	Previous malignancy/Rx	DMs (% cells)	HSRs	Reference
Bladder ca	1	65M	None	+	No	45
	1	62M	None	+	No	
	1	44M	None	No	+	
	1	84F	None	+	No	
Breast ca	1	(5 pt)	None	ND	+	46
Esophageal ca	Met	(1 pt)	None	ND	+	
Pharyngeal ca	1	(1 pt)	None	ND	+	
Breast ca	1	67F	None	+	No	47
Breast ca	1	46F	ND	+(7.5)	ND	48
	1	48F	ND	+(12)	ND	
	1	35F	ND	+(28)	ND	
	1	45F	ND	+(57)	ND	
	1	49F	ND	+(28)	ND	
	1	45F	ND	+(9)	ND	
	1	46F	ND	+(7)	ND	
	1	46F	ND	+(33)	ND	
	1	58F	ND	+(60)	ND	
	1	49F	ND	+(12.5)	ND	
	PE	43F	Hormonal	+(24)	ND	
	PE	55F	None	+(6)	ND	
	PE	56F	CT	+(54)	ND	
	PE	59F	CT	+(39)	ND	
	PE	60F	CT	+(10)	ND	
	AS	57F	None	+(27)	ND	
	AS	57F	CT	+(40)	ND	
Ovarian ca	AS	42F	CT	+(19)	ND	
	AS	48F	None	+(29)	ND	
	AS	49F	None	+(18)	ND	
	AS	51F	CT	+(12)	ND	
	AS	63F	None	+(19)	ND	
	AS	76F	None	+(18)	ND	
Cervical ca	AS	31F	None	+(29)	ND	
	AS	61F	None	+(1)	ND	
	AS	68F	None	+(60)	ND	
Cervical ca	AS	60F	ND	+(91)	ND	49
Ovarian ca	AS	78F	ND	+(100)	ND	
Uterine ca	AS	61F	ND	+(64)	ND	
Colon ca	1	34M	Colitis, steroids	+(100)	No	50
	1	(same pt)	RT, CT	+(100)	No	
Gastric ca	1	58F	None	+	ND	51
Colon ca	1	69F	None	+	ND	
Gastric ca	AS	43F	None	+	No	52
Glioma	1	F	ND	+(33)	ND	53
Glioma	1	ND	ND	+(7)	ND	54
Glioma	1	68M	ND	+(6.6)	ND	55
	1	39F	ND	+(9.7)	ND	
	1	66F	ND	+(11.8)	ND	
	1	63M	ND	+(5.4)	ND	
	1	49M	ND	+(80.2)	ND	
	1	58M	ND	+(81.8)	ND	
	1	69F	ND	+(100)	ND	
	1	71F	ND	+(7.4)	ND	
	1	40M	ND	+(91.6)	ND	
	1	50F	ND	+(7.4)	ND	
	1	69F	ND	+(22.1)	ND	
	1	51F	ND	+(2.7)	ND	
	1	56M	ND	+(22.2)	ND	

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Table 2. *(continued)*

Malignancy	Specimen	Age/Sex	Previous malignancy/Rx	DMs (% cells)	HSRs	Reference
Glioblastoma Multiforme	1	58F	None	+	No	56
	1	70M	None	+	No	
	1	64M	None	+	No	
	1	60M	None	+	No	
	1	46M	None	+	No	
	1	63M	None	+	No	
	1	71M	None	+	No	
	1	63M	None	+	No	
	1	42M	None	+	No	
	1	62F	None	+	No	
	1	54F	None	+	No	
	1	67M	None	+	No	
	1	54M	None	+	No	
	1	55M	None	+	No	
	1	64M	None	+	No	
	1	48F	None	+	No	
	1	44M	None	+	No	
	1	66F	None	+	No	
	1	72M	None	+	No	
	1	63F	None	+	No	
Anaplastic Astrocytoma	1	43F	None	+	No	
Anaplastic Gliosarcoma	1	46M	None	+	No	
Mixed glioma	1	75M	None	+	No	
Large cell Lung ca	1	62F	None	+(100)	No	57
Anaplastic bronchial ca	PE	58M	ND	+	ND	1
Medulloblastoma	BM	8F	None	+(90)	ND	58
Medulloblastoma	RE	16M	RT	+	No	59
Melanoma	LN	66M	CT	No	+	13
Melanoma	Met	29F	CT	+(90)	+(0.9)	60
Neuroblastoma	BM	1M	CT	+(70)	ND	61
	BM	2M	None	+(17)	ND	
Neuroblastoma	BM	3F	CT, RT	+(70)	ND	62
Neuroblastoma	BM	1F	CT	ND	+	63
	1	1M	CT, RT	ND	+	
Neuroblastoma	BM	2M	ND	+(100)	No	64
	BM	4M	ND	+	No	
	BM	5	ND	+	No	
Neuroblastoma	1	1F	No	+(100)	ND	65
Neuroblastoma	1	1M	None	+(100)	ND	66
	1	4F	RT, CT	+(22)	ND	
	1	1F	None	+(1/250)	ND	
Medulloblastoma	1	10M	None	+(100)	ND	
Embryonal sarcoma	1	3F	RT	+(60)	ND	
Ovarian ca	AS	58F	CT	+(2)	ABR	67
Ovarian ca	AS	65F	None	+(100)	No	68
Ovarian ca	1	46F	ND	+(100)	ND	69
Ovarian ca	AS	70F	ND	+(30)	ND	
Ovarian ca	AS	F	CT	+(1/212)	ND	70
(same pt after 9 mths additional CT)				+(13)	No	
Retinoblastoma	1	10 mths F	None	+(3)	ND	71
Retinoblastoma	1	3 mths	None	+(6.7)	No	72
	1	1 mth	None	+(84)	No	
	1	2 mths	None	+(14.5)	No	
Rhabdomyosarcoma	1	3F	RT	+	ND	73

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Table 2. *(continued)*

Malignancy	Specimen	Age/Sex	Previous malignancy/Rx	DMs (% cells)	HSRs	Reference
Rhabdomyosarcoma	LN	16M	RT	+(100)	No	74
Seminoma/teratoma	1	41M	None	+(76)	ND	75
Malignant teratoma	1	30M	None	+(12)	ND	
Primitive neuroectodermal tumor	1	7M	None/None	+	No	76
Primitive neuroectodermal tumor	1	5M	None/None	+	No	
Primitive neuroectodermal tumor	1	2M	None/None	+	No	
Retroperitoneal Teratoma	Met	ND/M	ND/CT	No	+	77
Testicular teratoma	Met	ND/M	ND/CT	No	+	
Testicular Embryonal Carcinoma	Met	ND/M	ND/None	+(12)	+(100)	
Medicastinal Seminoma	1	ND/M	ND/None	+(83)	+(100)	

ABR, abnormal banding regions; AS, ascites; BM, bone marrow; ca, cancer; CT, chemotherapy; LN, lymph node; Met, metastasis; ND, not determined (or not stated in the original paper); PE, pleural effusions; pt, patient; 1, primary tumor; RE, recurrence; RT, radiation therapy.

tumors are listed in Table 2. This table is comprised of 125 patients with 23 different histologic types of malignancies. Neuroblastomas and gliomas are the most frequently described tumors with DMs. As with the directly examined leukemias, in these specimens DMs predominate. Of the 125 cases, 108 report only DMs, 13 report only HSRs, and four reported DMs plus HSRs (or ABRs). The percentage of cells in each specimen which contain DMs ranged from 1 to 100% (median = 28%) (Figure 2A). Papers which would have described both DMs and HSRs (totally evaluable reports), if present (for solid tumors), found 45 cases with DMs alone, four with HSRs alone, and four with both (Figure 2B).

Among the cases in Table 2, none had previous malignancies. Previous chemotherapy or radiation therapy had been given in 22 cases. Interestingly, of the 17 cases of HSRs described in fresh specimens, seven of the malignancies were from previously treated patients.

As can be seen in Tables 1 and 2, a wide variety

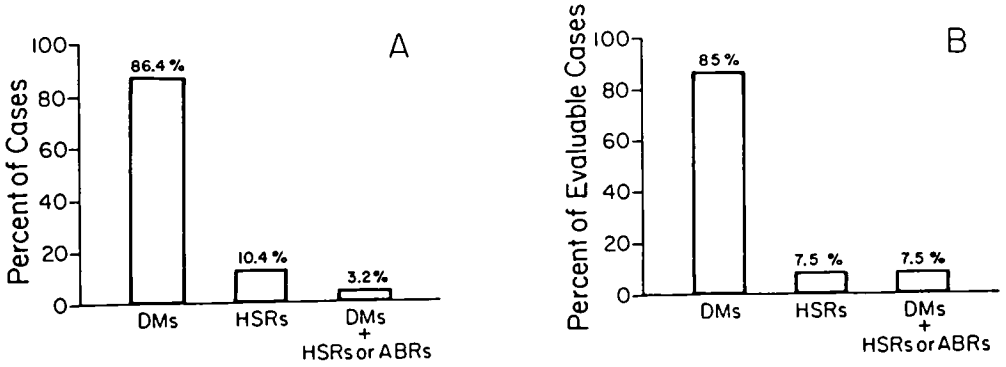

Figure 2. (A) Per cent of solid tumor cases examined directly from patients with DMs, HSRs, or both (total cases reviewed). Of 125 cases reviewed 108 (86.4%) had DMs only, 13 (10.4%) had HSRs only, and 4 (3.2%) had DMs and an HSR reported. (B) Per cent of totally evaluable (see text) solid tumor cases examined directly from patients with DMs, HSRs, or both. Of 53 evaluable cases 45 (85%) had DMs only, 4 (7.5%) had HSRs only, and 4 (7.5%) had DMs and an HSR reported.

Table 3. Human derived cell lines with DMs or HSRs

Malignancy	Cell line	DMs (% cells)	HSRs	Reference
AML (M2)		+	ND	78
Promyleocytic	HL-60	No	ABR	79, 80
Leukemia		+	No	
Promyelocytic	HL-60	+	ND	81
Leukemia				
Bloom's syndrome	GM 1492	+	No	82
Breast ca, PE	MDA-MB			3
	134	+(10)	ND	
	157	+(4)	ND	
	175	+(10)	ND	
	231	+(42)	ND	
	253	+(14)	ND	
	309	+(20)	ND	
	330	+(8)	ND	
	415	+(26)	ND	
From solid tumors	BT-474	+(34)	ND	
	SW-527	+(44)	ND	
	SW-613	+(80)	ND	
	MD-MB-361	+(42)	ND	
From lung Met	SW-732	+(80)	ND	
Breast ca	MDA-MB-321	ND	+	83
Breast ca	MCF-7	+	ND	84
Breast ca		+	ND	48
		+	ND	
		+	ND	
		+	ND	
Carcinoma	NCI-H548	+	No	85
	NCI-H630	+	+	
	NCI-H684	+	+	
	NCI-H508	+	No	
	NCI-H747	+	No	
	SNU-C1	+	No	
	SNU-C2A	+	No	
	SNU-C5	+	No	
	NCI-H716	+	+	
Cervical ca	HeLa F-1000	+	No	86
	HeLa S3	+	No	
	HeLa TCH-3753	+	No	
Colon ca (neuroendocrine derived)	COLO-320	+	+	87, 88, 89
Colon ca	VAC0206	+	No	90
Medulloblastoma	D-341	+	No	59
Melanoma	MeWo	ND	+	91, 92, 93, 94, 95
Mesothelioma		No	+	96
Myeloma	U-1996	No	+	97
Neuroblastoma	IMR	ND	+	98
Neuroblastoma	CHP-134	No	+	99, 100, 101, 102
	NMB	No	+	
	CHP-126	+	+	
Neuroblastoma	Y79 NHTC	ND	+	103, 104
Neuroblastoma	IMR-32	No	+	105, 106, 107
	MC-186	+	No	
	NB-9	+	No	
	NB-16	+	No	
	NB-19	No	+	102, 103, 104

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Table 3. *(continued)*

Malignancy	Cell line	DMs (% cells)	HSRs	Reference
	LA-N-1	No	+	
	LA-N-5	+	No	
Neuroblastoma	SH-SYSY/VCR	+	No	108
Neuroblastoma	SK-N-BE	+	No	109
	BE (2)-6	No	+	
	NAP (D)	+	No	
	NHP (H)	No	+	
	CHP-234	+	No	
	SMS-KAN	+	No	
	SMS–KCNR	+	No	
Neuroblastoma	Kelly	No	+	110
	NGD	No	+	
	NLF	No	+	
	NMB	+	+	
	MCN-1	+	No	
Neuroblastoma	TR14	+	+	111
Neuroblastoma	CHP-382	No	+	112
	CHP-382JK	No	+	
Neuroblastoma	NB-56	No	+	113
	NB-76	+	No	
	NMB-7	+	+	
Neuroblastoma	MC-NB-1	+	No	114
Neuroblastoma		+	No	115
Neuroblastoma	SMS-KANR	No	ABR	116
	SMS-MSN	+	No	
Ovarian ca		+	+	117
		+	No	
		+	+	
		No	+	
Ovarian ca		+	No	48
Rhabdomyosarcoma		+	ND	118
Rhabdomyosarcoma	BG	No	+	119
	DND	No	+	
Salivary gland tumor		+	No	120
Small cell lung ca	PE			121, 122
	NCI-H60	+(20)	No	
	NCI-H69	+(30)	No	
	NCI-H832	No	+(96%)	
	NCI-N417	No	+	
Small cell lung ca	DMS-53	+	ABR	123
	DMS-55	+	No	
	DMS-79	+	ND	
	DMS-106	+	No	
	DMS-114	+	ABR	
	DMS-139	+	No	
	DMS-153	+	No	
	DMS-319	+	No	
	DMS-187	+	No	
	DMS-235	+	No	
Small cell lung ca	NCI-H249P	+	ND	124
Small cell lung ca	U-1906	+	No	125
	U-2020	+	No	
	H-82	No	+	
Small cell lung ca	SCLC-22H	+	+	126
Large cell lung ca	U-181	No	+	125
T-cell lymphoma/leukemia		+	No	79
Thyroid ca		+	ND	127
Breast ca	21	+(5)	+(5%)	128

ABR, abnormal bonding region, AML, acute myelogenous leukemia; ca, cancer; Met, metastasis; ND, not determined (or not stated in the original paper); PE, pleural effusion.

of malignancies have been shown to have DMs. The incidence of DMs or HSRs, however, is much more difficult to estimate. The series in the tables vary from describing DMs or HSRs as an interesting finding in an isolated case to suggesting that these structures occur frequently. Marinello, for example, found DMs in 40 cases when he studied successive bone marrows from 320 patients with hematologic disease.[35] Li described DMs in 5 of 110 patients with acute myelogenous leukemia.[30] In another series of sequential malignant gliomas, 18 of 54 had DMs.[56] Clearly, a retrospective review cannot define the incidence of these abnormalities.

DMs and HSRs in cell lines

There have also been many reports of DMs and HSRs in human cell lines. Table 3 lists 109 cell lines in which we have found at least one of these abnormalities reported. There are 20 different conditions represented, all except two are malignancies. A benign salivary gland tumor cell line and a line derived from a patient with Bloom's syndrome both contained DMs without HSRs. Neuroblastoma was the most frequently reported malignancy, accounting for over a third of the cell lines. Breast cancer and small cell lung cancer were also reported frequently. The frequency of small cell lung cancer lines is in contrast to the absence of reports of small cell lung cancer specimens obtained directly from patients.

Among all of these cell lines, 66 report only DMs, 29 report only HSRs, and 14 report both DMs and HSRs (or Abnormal Banding Regions, (ABRs)) (Figure 3A). Papers which would have included both abnormalities, if present (totally evaluable cases), listed 43 lines with DMs alone, 24 with HSRs alone, and 14 with both (Figure 3B).

Comparison of tumors examined directly from patients vs from human tumor cell lines

Figure 4 summarizes the data for the 82 totally evaluable cases of karyotypes performed on tumors taken directly from patients (29 leukemias + 46 solid tumors) (Figure 4A) vs the data for the 81 totally evaluable cases of karyotypes performed on cell lines (Figure 4B). There are striking differences in the frequency of DMs and HSRs in tumors examined cytogenetically directly from patients vs tumors examined cytogenetically after propagation as cell lines. DMs are more commonly described than HSRs in specimens examined directly from patients. In cell lines which often have been exposed to the selective pressures of tissue culture, DMs occur less frequently, and HSRs occur more frequently than they do in primary human tumor specimens.

Discussion

We have reviewed the published cytogenetic analyses of tumors taken directly from patients to determine the percentage of primary human tumors which have extrachromosomal evidence of gene amplification (in the form of DMs) vs the percentage of those with intrachromosomal evidence of gene amplification (in the form of HSRs). In the total analysis of 200 leukemias, myelodysplas-

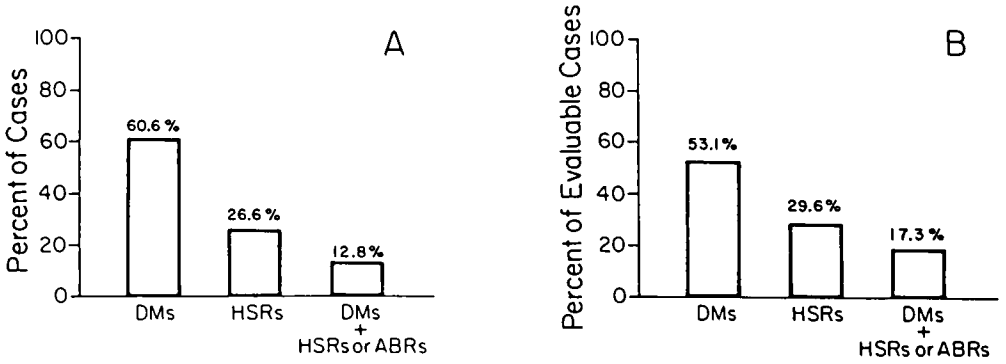

Figure 3. (A) Per cent of cell lines reviewed with DMs, HSRs, or both (total cases reviewed). Of 109 total cases reviewed 66 (60.6%) had DMs only, 29 (26.6%) had HSRs only, and 14 (12.8%) had DMs and an HSR (or ABR). (B) Per cent of cell lines reviewed (evaluable cases—see text) with DMs, HSRs, or both. Of 81 evaluable cases, 43 (53.1%) had DMs only, 24 (29.6%) had HSRs only, and 14 (17.3%) had DMs and an HSR (or ABR).

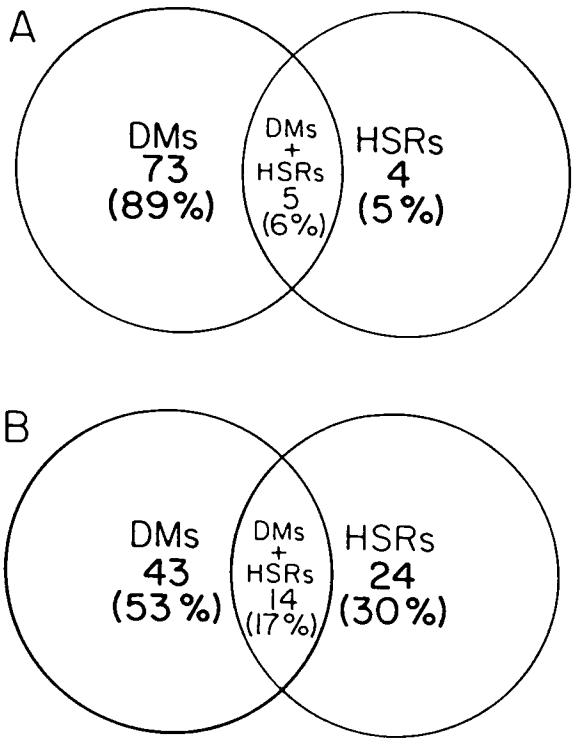

Figure 4. (A) Summary of DMs or HSRs found in evaluable karyotypes performed on 82 tumors taken directly from patients (29 leukemias + 53 solid tumors). Note predominance of cases with DMs (73 cases—89% of evaluable karyotypes). (B) Summary of DMs or HSRs found in evaluable karyotypes performed on 80 human tumor cell lines. Note cell lines have a greater incidence of HSRs.

tic, and solid tumor cases we found 91% had DMs only, 6.5% had HSRs only, and 2.5% had evidence of both. This is in contrast to the total of 109 cell lines in which karyotyping revealed 60.6% had DMs only, 26.6% had HSRs only, and 12.8% had evidence of both.

An assessment was also made of totally evaluable cases where we could make sure that if DMs or HSRs were present they would definitely have been reported. In the analysis of 82 evaluable cases of karyotypes performed directly from patients (29 leukemias and 53 solid tumors) 89% had DMs only, 5% had HSRs only, and 6% had evidence of both. This is in contrast to the 81 evaluable cases of karyotypes performed on cell lines of which 53% had DMs only, 30% had HSRs only, and 17% had evidence for both.

The above data indicate that DMs are the predominant cytogenetic manifestation of gene amplification in tumors taken directly from patients. These data also indicate that there may be a shift in the cytogenetics of tumor cells (in particular the amplification units) from an extrachromosomal site to an intrachromosomal site with establishment and passage of cells *in vitro*. Other investigators have noted that when cell lines are followed in culture the DMs tend to be lost, especially in the absence of selective pressure, whereas HSRs are preserved.[61] These findings are consistent with the model whereby amplified sequences are initially localized *in vivo* to an extrachromosomal compartment (episomal DNA and DMs or both).[6–11] With establishment of culture and passage *in vitro*, cells containing HSRs are selected in the process of clonal growth. It is quite likely, therefore, that studies of gene amplification carried out on established cell lines may not be optimal since selective processes of culturing may obscure the identity of the initial products of amplification.

As outlined in the Introduction, there is now evidence that extrachromosomally located copies of amplified drug-resistant genes or oncogenes can be eliminated from tumor cell lines by exposure of those lines to relatively low concentrations of hydroxyurea.[15–18] In this study we have found that double minutes (extrachromosomal DNA) are a more common cytogenetic manifestation of gene amplification in primary human tumors than are HSRs (intrachromosomal location for the amplified DNA). The findings that DMs predominate as the form of gene amplification in patients' tumors indicate that DMs in cells are potential targets for therapeutic approaches. Elimination of the extrachromosomal DNA *in vivo* might reverse drug resistance or modulate tumor progression.

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