British Journal of Cancer (1999) **80**(5/6), 862–873

Article no. bjoc.1998.0433

Comparative genomic hybridization and chromosomal instability in solid tumours

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Keywords: comparative genomic hybridization; solid tumour genetics; chromosomal instability

The rational development of new diagnostic or prognostic tumour markers and the identification of novel cellular targets for anti-cancer chemotherapy relies on a more definitive understanding of tumour biology. Classical approaches using cellular pharmacology, and more recently molecular pharmacology, have led to the discovery of a number of growth factors and their receptors as well as other proteins which has resulted in novel therapies (e.g. inhibitors of epidermal growth factor receptor tyrosine kinase) and prognostic markers (e.g. oestrogen receptor levels in breast cancer) (Levitzki et al, 1995; Dowsett et al, 1997). Using classical metaphase cytogenetic techniques, many chromosomal aberrations have been identified in human cancer cell lines and primary culture of haematological malignancies. This chromosomal information has facilitated identification of a number of impor–tant genes associated with tumorigenesis (e.g. loss of chromosomal material on 13q led to identification of tumour suppressor gene RB1; Vogel, 1979). However, the use of metaphase cytogenetic analysis has been limited in solid tumours, mainly due to the difficulties in growing primary cultures in which to generate tumour metaphase chromosomes. However, this changed with the development of comparative genomic hybridization (CGH) and its ability to globally assess the genome of solid tumours for areas of loss and/or gain without the need for tissue culture (Kallioniemi et al, 1992; Forozan et al, 1997; Ried et al, 1997). CGH involves a competitive in situ hybridization of fluorescently labelled tumour DNA and healthy control DNA to normal metaphase chromosomes (Figure 1). Computer-assisted fluorescence microscopy is then used to assess the intensity of fluorochrome across each human chromosome. The differences in tumour and control fluorescence intensity along each chromosome on the reference metaphase spread are a reflection of the copy number changes of corresponding sequences in the tumour DNA. If chromosomes or chromosomal subregions are present in identical copy number within both the tumour and the normal DNA, an equal contribution from each fluorochrome is seen. However, a change in the fluorescent signal is seen if certain chromosomal subregions are gained or lost in the tumour DNA (Figure 1). The intensity of this signal is proportional to the amount of gain and loss seen for each region in the tumour DNA (Kallioniemi et al, 1992; Forozan et al, 1997). Regions with a high level of heterochromatin and centromeric regions are not informative with CGH. CGH data for the p regions of acrocentric chromosomes (e.g. 13p, 14p and 15p) must be interpreted with caution as repetitive sequences in these regions can affect the efficiency of competitive hybridization. With current technology, CGH has a theoretical limit of detection for gain and loss of genetic material of 5–10 Mb. However, gain of DNA in regions as small as 50 kb have been described in situations where high level amplification has occurred (Ried et al, 1997).

Initial studies with CGH were restricted to DNA prepared from fresh or snap-frozen tumour material. More recently, technical advances have allowed the extraction of DNA from formalin-fixed paraffin-embedded sections through the use of degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) (Isola et al, 1994; Kuukasjarvi et al, 1997). The DOP-PCR technique allows genome-wide amplification of tumour DNA from nanogram quantities to the micrograms needed for CGH, and has enabled retrospective analysis of genomic loss and gain to be performed using DNA from archival material.

Although CGH analysis has been performed in a wide variety of adult and paediatric tumours, these results have not been extensively interpreted in the context of the CGH findings from other tumour types. In this review, the results of CGH analysis in 27 tumour types are evaluated to identify regions of loss or gain which are common to all malignancies as well as those which are specific for a given tumour type or tumour subtype. In addition, the degree of overall genomic instability for specific tumour types has been assessed.

REVIEW OF THE LITERATURE

The Institute for Scientific Information (ISI) database from March 1992 to August 1998 identified 100 papers which described CGH findings in 2210 solid tumours of 27 cancer types (Appendix). This included common tumours (colon, breast, lung), gender-specific tumours (ovarian, cervix, testicular, prostate), paediatric tumours (neuroblastoma, rhabdomyosarcoma) and less common tumours (brain, renal, uveal melanoma). For each paper, the patterns of loss and gain in the p and q arms of each chromosome were recorded separately. Such an approach may not always be sufficient, as variation in subregions of the same chromosomal arm could be masked in some cases. However, a narrower definition for regions of gain and/or loss was not possible due to differences in the way CGH results have been presented in the

Received 17 July 1998
Revised 28 September 1998
Accepted 21 October 1998

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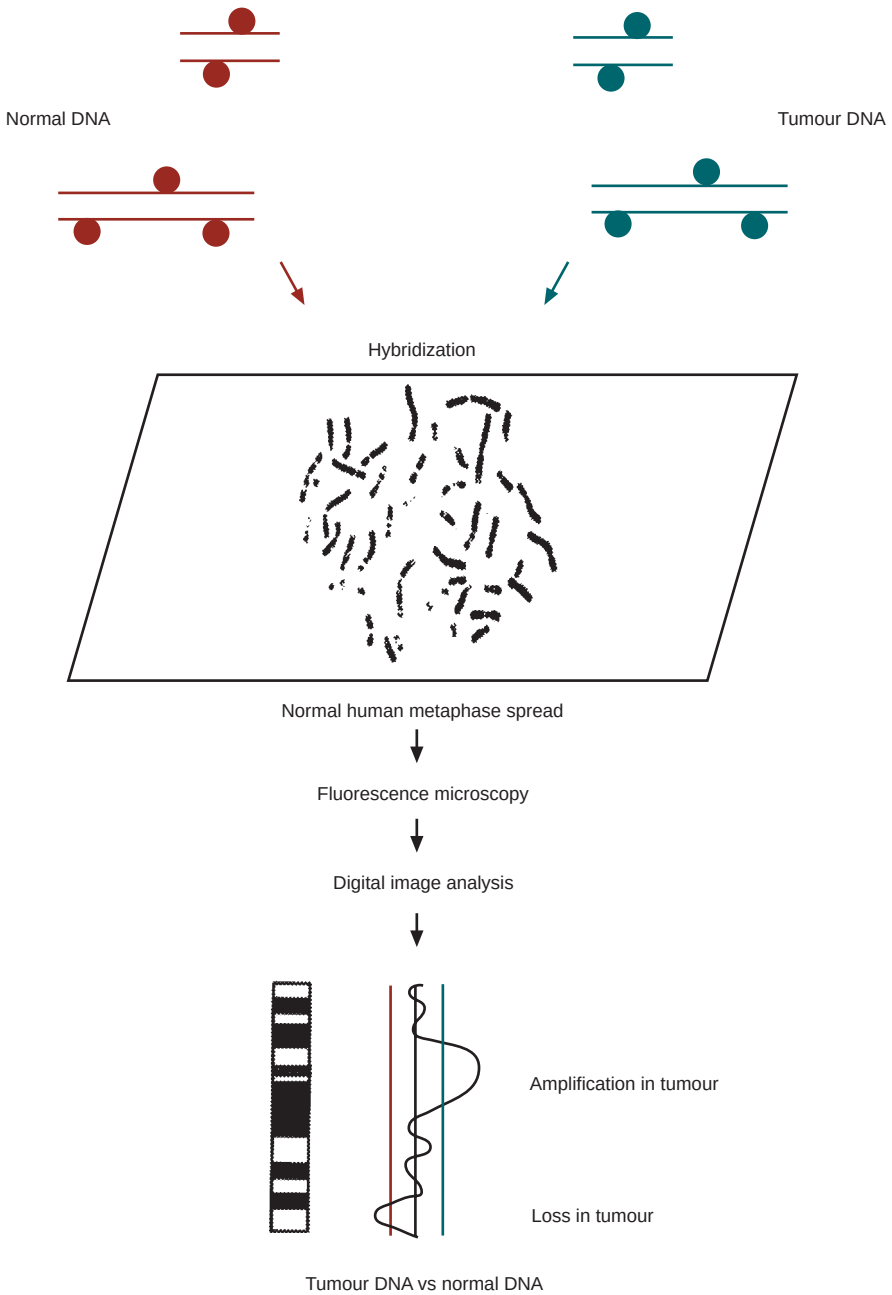

Figure 1 A typical CGH experiment. Fluorescently labelled tumour DNA and reference DNA are competitively hybridized to donor human chromosomes. Using fluorescent microscopy the level of signal from the fluorescent DNA is assessed for each chromosome. For each chromosome a profile of the level of fluorescence is generated on CGH interpreting software. In most cases at least 10 chromosomes are assessed and an average of the fluorescence is generated. This allows regions of loss and gain that are consistently changed to be detected for a particular tumour sample

literature. Studies of CGH in patients with leukaemia, lymphoma or studies with incomplete details of results for individual chromosomes were not included in this review. Cell line data were not included due to the difficulty in differentiating between initial chromosomal aberrations and those 'acquired' during cell culture. The frequency of overall loss or gain for each chromosome arm was determined by pooling the data from all tumours, from a given tumour type and from specific tumour subtypes.

PATTERNS OF CHROMOSOMAL LOSS AND GAIN

Solid tumours

The frequency of loss or gain for each chromosome arm was determined for all the solid tumours by pooling the data found in the literature for 2210 tumours (Table 1). Gain of chromosomal material was found more frequently than loss among the solid

Table 1 Loss and gain for each chromosomal arm when available CGH data from 2210 tumours (including 27 different solid tumour types) were pooled

Chromosomal region	Total tumour *n* = 2210	Gain (%)	Chromosomal region	Total tumour *n* = 2210	Loss (%)
8q + gains	616	27.7	13q – losses	363	16.3
1q + gains	558	25.1	9p – losses	357	16.1
7q + gains	513	23.1	8p – losses	333	15
7p + gains	477	21.5	10q – losses	304	13.7
17q + gains	412	18.5	3p – losses	297	13.4
3q + gains	365	16.4	4q – losses	297	13.4
20q + gains	344	15.5	6q – losses	296	13.3
5p + gains	292	13.2	17p – losses	260	11.7
12q + gains	290	13.1	18q – losses	245	11
12p + gains	277	12.5	1p – losses	226	10.2
11q + gains	252	11.3	11q – losses	218	9.8
6p + gains	246	11.1	5q – losses	206	9.1
20p + gains	223	10	10p – losses	202	9.1
19q + gains	223	10	16q – losses	196	8.8
2p + gains	214	9.6	4p – losses	188	8.5
13q + gains	205	9.2	22q – losses	184	8.3
19p + gains	203	9.1	14q – losses	183	8.2
1p + gains	201	9	9q – losses	170	7.7
14q + gains	201	9	11p – losses	167	7.5
2q + gains	198	8.9	15q – losses	161	7.2
17p + gains	179	8.1	2q – losses	153	6.8
16p + gains	176	7.9	Xp – losses	152	6.8
8p + gains	175	7.9	Xq – losses	126	5.7
15q + gains	174	7.8	21q – losses	122	5.5
5q + gains	168	7.6	Y – losses	122	5.5
6q + gains	164	7.4	18p – losses	119	5.4
9q + gains	156	7	19p – losses	112	5
18p + gains	153	6.9	17q – losses	105	4.7
16q + gains	140	6.3	3q – losses	102	4.6
18q + gains	136	6.1	12q – losses	98	4.4
22q + gains	133	6	19q – losses	96	4.3
10p + gains	131	5.9	1q – losses	89	4
Xq + gains	129	5.8	6p – losses	84	3.8
4q + gains	118	5.3	16p – losses	83	3.7
10q + gains	117	5.3	5p – losses	83	3.7
9p + gains	116	5.2	2p – losses	82	3.7
Xp + gains	115	4.7	8q – losses	64	2.9
3p + gains	104	4.7	7q – losses	56	2.5
21q + gains	101	4.5	20q – losses	53	2.4
11p + gains	97	4.4	20p – losses	53	2.4
4p + gains	95	4.3	12p – losses	52	2.3
Y + gains	55	2.5	7p – losses	50	2.3
14p + gains	24	1.1	22p – losses	36	1.6
21p + gains	22	1	15p – losses	21	0.9
13p + gains	17	0.8	14p – losses	10	0.5
15p + gains	9	0.4	13p – losses	9	0.4
22p + gains	6	0.3	21p – losses	3	0.1
Total gains	9320/2210	4.2 per tumour	Total losses	6988/2210	3.1 per tumour

tumours (mean 4.2 gain per tumour vs 3.1 loss per tumour). A variable pattern of chromosomal gain was observed, with the highest frequency of gain found in 8q (27.7%) and 1q (25.1%) (Table 1). This contrasts with chromosome 22p (0.3%) and 15p (0.4%) where gain of chromosomal material was rarely observed (Table 1). The most common regions of chromosomal loss were found on 13q (16.3% of all tumours), 9p (16.1%) and 8p (15.0%) (Table 1). Loss of chromosomal material was rarely seen on chromosome 21p (0.1%), 13p (0.4%) and 14p (0.5%). From Figure 2 it can be seen that levels of loss and gain are not uniform across all chromosomal regions. Certain chromosomal regions, such as 8q, are often gained (27.7%) but rarely lost (2.9%). Similarly, loss in chromosome 4q was more common (13.4%) than gain (5.3%). This pattern was not seen for all chromosomes, with loss of 13q (16.3%) only 1.8 times more common than gain (9.2%). Patterns of nearly equal frequency of loss and gain were also observed for chromosomes 14q (9% gain vs 8.2% loss) and 15q (7.8% gain vs 7.2% loss). However, this does not take into account the specific region of a chromosomal arm to which the genetic loss or gene amplification in solid tumours is mapped. It also does not account for tumour-specific patterns of chromosomal gain and loss (Table 2), where the same chromosomal arm is rarely lost and gained to an equal extent for a particular tumour type.

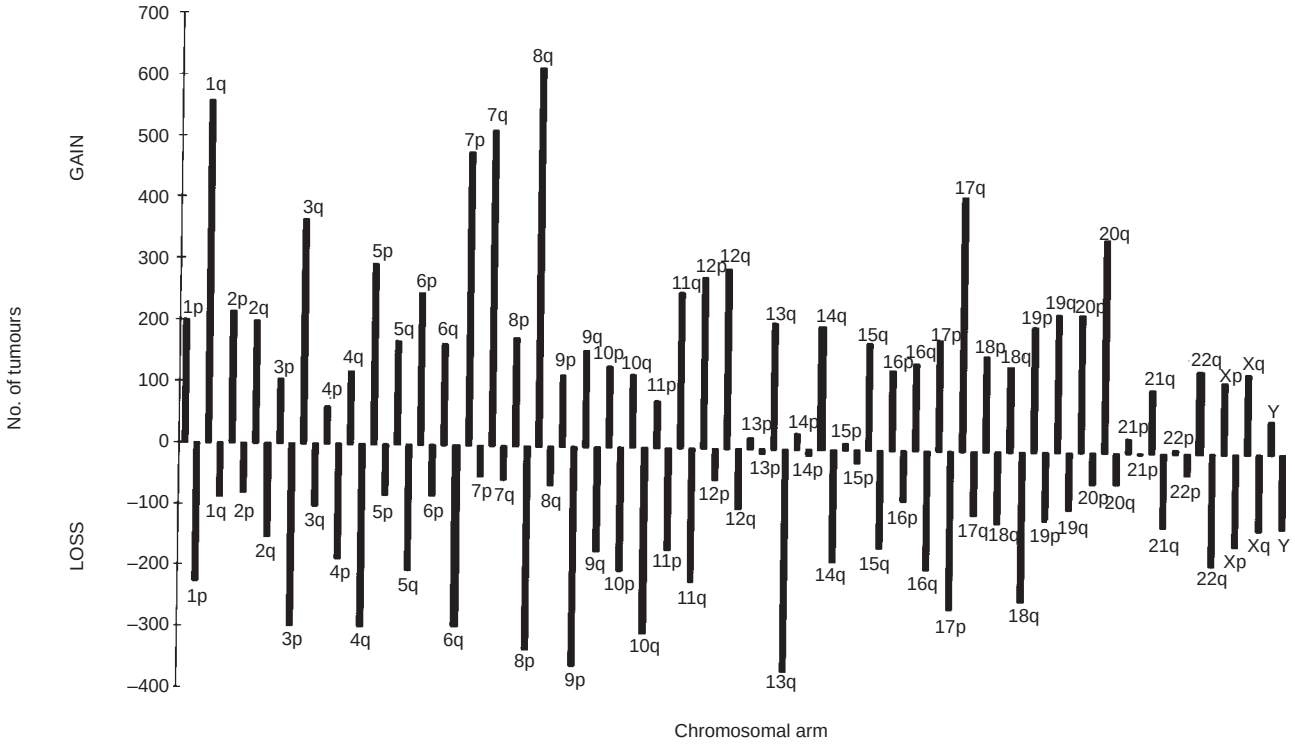

Figure 2 The overall number of gains and losses detected in 2210 solid tumours from 27 different tumour types

Specific tumour types

The frequency of chromosomal loss and gain varied between the individual tumour types, ranging from multiple regions per tumour (average gains: head and neck 12.2 per tumour, testicular 8.2 per tumour; loss: liver 7.5 per tumour, prostate 4.5 per tumour) to relatively rare events (average gains: neuroblastoma 0.5 per tumour, Wilms' 1.6 per tumour; loss: sarcoma 0.8 per tumour, Wilms' 1.3 per tumour (Table 3). The specific chromosomal regions of loss and gain differ substantially between specific tumour types. For example, gain in chromosome 12p occurred in 96.3% of testicular cancers and 0% of renal cancers (Table 2). New information on chromosomal loss or gain (Table 1) can be further specified amongst the various tumour types. For example, gain in chromosome 8q occurred in 27.7% of all tumours evaluated. However, on closer examination, frequency of 8q gain was high in tumours of the testis (40.7%), ovary (42.8%) and endometrium (45.5%), but was rarely found in renal tumours (1.3%) and neuroblastoma (3.0%). There is no chromosomal arm which demonstrated a consistent pattern of gain for all tumour types. Similar findings were demonstrated for chromosomal loss. For instance, 9p was lost in 16.1% of all tumours, but varied from a high frequency event (cutaneous melanoma 58.2%, pancreas 50.1%, brain 36.3%) to low (colon 7.5%, gastric cancer 7%) depending on the tumour type (Table 2).

Specific tumour subtypes

For several tumours, CGH analysis was available for multiple histological subtypes (Table 4). This allowed assessment of both the frequency at which loss and gain occurred and the extent to which each specific chromosomal arm is involved for each subtype.

Colon

Information on genomic alterations in colon cancer was available for low- and high-grade adenoma, primary carcinomas, liver metastases, and also carcinomas for which replication error repair status was known (Table 4). Ried et al (1995) found the frequency and degree of genetic aberrations increases with progression from low-grade adenoma through high-grade adenoma to carcinoma (Table 4). For example, gain in chromosome 7p was 7.1% in low-grade adenoma, 33.3% in high-grade adenoma and 50% in carcinoma. Similarly, gain in chromosome 20q was not detected in low-grade adenoma, but was at 33.3% and 75% in high-grade adenoma and carcinoma respectively. The frequency of alterations also increased with tumour progression: 3/47 chromosomal arms in low-grade adenoma, 21/47 high-grade adenoma, 32/47 carcinomas. A separate study by Paredes-Zaglul et al (1998) comparing primary carcinomas and liver metastases from patients with colorectal cancer found that the frequency of alteration remained constant at ~ 35/47 chromosomal arms between these two stages. However, a change was noted in the extent to which these arms were involved. The most obvious change being the increase in loss of genetic material between primary tumour and liver metastases. For example, loss at 8p was 30% in primary carcinomas compared with 80% in metastases. Similarly, loss of 18q was found in 50% of primary cases, but 90% of liver metastases. Changes in gain did not always follow the same pattern seen for loss. An increase in genetic instability was seen for some chromosomal regions in the transition from primary to metastases (e.g. 13q was gained in 30% of primary tumours compared with 50% in metastases). However, this was not the case for other regions, such as 12q, which was gained in 20% of primary carcinomas, but was normal in liver metastases. A difference in genetic instability was also seen between tumours with intact mismatch repair genes compared to

Table 2 The ten most frequently lost and gained chromosomal regions for each tumour type studied. Percentage of tumours with alterations of the specific chromosomal arm are shown. Fewer than ten altered arms were found in MCC.

CR	GC	CR	H&N	CR	Pancreas	CR	Colon	CR	Prostate	CR	Testicular	CR	Breast	CR	Ovary	CR	Endo	CR	Cervical	CR	C mel	CR	MCC	CR	U mel	CR	Renal
Top 10 losses																											
Y	22.1	3p	53.2	9p	50.1	18q	31.3	8p	42	13q	33.3	17p	18.2	8p	26.6	15q	21.2	3p	50	9p	58.2	10q	33.3	3p	45.5	3p	39.1
4q	16.2	5q	42.6	18q	47.8	17p	22.5	16q	39	4q	26	11q	12.3	17p	16.7	18q	12.1	2q	33.3	10q	44.8	11q	33.3	3q	45.5	13q	27.2
19p	14.7	4p	40.4	6q	33.3	18p	15	16q	39	21q	26	8p	11.7	16q	16.3	4q	12.1	6q	26.6	10p	28.3			6q	45.4	8p	20.5
1p	13.2	4q	32	21q	30.4	4p	12.5	16q	39	6q	22.2	6q	11.2	18q	14.8	16q	9.1	13q	26.6	9q	25.4			9p	27.3	6q	23.2
9p	10.3	13q	30	3p	26.1	17q	12.5	13q	36	5q	18.5	13q	10.7	17q	12.8	9p	9.1	4p	23.3	6q	25.3			11p	27.3	1q	17.9
5q	8.8	9p	27.7	4q	21.7	8p	11.3	6q	23	Xq	18.5	4p	9.6	5q	9.9	8p	9.1	4q	23.3	8p	22.3			12p	27.3	10q	17.2
6p	7.4	6q	23.4	8p	20.3	5q	8.8	17p	23	5q	14.8	18q	9.1	Xp	9.4	Xp	6.1	8p	23.3	5q	13.4			1p	18.2	14q	17.2
10q	7.4	2q	21.3	17p	20.3	1p	7.5	2q	11	18p	14.8	18p	8	Xq	8.9	4p	6.1	9p	20	1p	11.9			16q	18.2	8p	15.9
11q	7.4	16q	21.3	11q	17.4	9p	7.5	9q	8	18q	14.8	11p	7.5	19p	7.4	5q	6.1	11q	20	3q	11.9			Xp	18.2	9p	15.9
4p	5.9	1p	17	13q	17.4	4q	6.3	17q	8	4p	11.1	3p	6.4	19q	7.4	13q	6.1	14q	20	17p	5.9			Xq	18.2	2p	14.6
Top 10 gains																											
17q	42.6	3q	61.7	20q	47.8	20q	33.8	8q	43	12p	96.3	1q	52.9	8q	42.8	1q	54.5	3q	76.6	8p	41.8	1p	66.6	8q	63.6	17q	23.2
8q	30.9	5p	59.6	8q	34.8	7p	23.8	Xp	26	Xp	60	8q	38.5	20q	38	8q	45.5	1q	46.6	7q	37.3	1q	66.6	6p	54.5	7p	18.5
20q	30.9	2q	51.1	11q	29	13q	27.5	7q	23	8q	40.7	17q	19.8	3q	33	10p	21.2	5p	30	7p	35.8	6p	66.6	8p	18.2	5q	17.2
17p	27.9	11q	44.7	7p	27.5	7q	22.5	Xq	20	7q	30	11q	19.8	1q	23.6	10q	15.2	6p	26.6	8q	30	6q	66.6	17p	18.2	1q	16.6
7p	26.5	17p	44.7	7q	27.5	8q	23.8	7p	18	8p	30	16p	13.9	1p	20.2	13q	21.2	20p	23.3	6p	30	18q	66.6	17q	18.2	7q	15.9
20p	26.5	17q	44.7	20p	27.5	20p	17.5	8p	14	7p	25.9	3q	10.2	7q	19.7	5p	27.3	8q	20	1q	25.4	20p	66.6	7p	9.1	17p	15.2
1q	23.5	15q	42.6	14q	26.1	9q	13.8	3q	12	20q	22.2	22q	10.2	2q	18.7	6p	33.3	9p	20	17q	15	20q	66.6	9p	9.1	19p	12.6
9q	19.1	20q	42.6	18p	26.1	9p	7.5	18q	12	Xq	22.2	Xq	9.6	11q	18.2	11q	24.2	15q	20	20q	15	8p	33.3	9q	9.1	16p	11.3
13q	19.1	7q	40.4	3q	20.3	1q	6.3	2q	10	2p	18.5	8p	5.3	6p	16.7	7q	21.2	19q	20	2p	13.4	8q	33.3	11p	9.1	22q	8.6
3q	17.6	12q	40.4	12p	17.4	13p	6.3	16p	9	2q	18.5	3q	5.3	13q	11.3	6q	9.1	Xq	20	5p	11.9	9p	33.3	11q	9.1	20q	7.9

Top 10 losses

CR Bladder		CR Wilms'		CR Sarcoma		CR Rehab		CR Lung		CR Liver		CR Neuro		CR Brain		CR GE*		CR Para		CR Pit		CR N Endo		CR GISTS	
9q	33.3	4q	50	1q	15.6	16q	20.8	3p	32.7	4q	81.4	1p	27.9	10q	45.5	5q	53.3	11q	34	18p	17.4	11q	30	14q	75
9p	25	9p	50	13q	8.4	10p	20.3	4q	25.5	8p	74.4	3p	22.5	9p	36.3	4p	46.7	11p	28.3	19p	13	16p	30	15q	34
8p	20.1	3q	37.5	1p	7.2	15q	16.7	17p	22.4	6q	46.5	4p	22.4	10p	33.5	4q	40	1p	18.9	19q	13	18p	25	22q	34
11q	20	3p	25	2q	6.6	16p	16.7	10q	22.3	13q	41.9	11q	21.7	13q	28	9p	20	13q	18.9	17p	8.7	1p	20	1p	28.1
7q	18.3	4p	25	11q	5.4	10q	12.5	10p	21.8	Xq	41.9	3q	19.4	22q	19.3	18q	20	15q	17	17q	8.7	9p	20	13q	18.8
2q	17.7	11p	25	9p	4.8	11q	12.5	5q	21.7	1p	35	4q	18.6	14q	16.9	3p	13.3	1q	11.3	18q	8.7	17p	20	3p	12.5
Y	16.6	11q	25	4q	4.2	14q	12.5	13q	21.4	Xp	34.9	10q	14	6q	16	5p	13.3	6q	11.3	1p	4.3	19p	15	8p	6.3
11p	14.6	17p	25	3q	3	1q	8.3	9p	21	17p	30.2	21q	21.7	18q	12.1	6q	13.3	9p	11.3	2q	4.3	19q	15	1q	3.1
10q	12.5	19q	25	8p	3	2q	8.3	20q	13	16p	25.6	8p	11.6	Xq	10.2	1p	6.7	9q	9.4	8q	4.3	4q	10	3q	3.1
16q	11.5	20p	25	10p	3	4p	8.3	8p	12.3	14q	20.9	11p	11.6	19p	7.5	2p	6.7	2p	5.7	13q	4.3	6q	10	5q	3.1
Top 10 gains																									
8q	17.7	12p	62.5	1q	13.8	12q	54.2	5p	40.7	8q	70	7p	40.3	7q	52	7q	60	16p	11.3	5q	26.1	14q	55	5q	25
17q	14.6	12q	62.5	1p	13.1	2p	50	3q	40.2	1q	67.4	7q	38	7p	51	8q	60	19p	9.4	4q	21.7	5p	50	5p	21.9
3q	12.5	7p	37.5	8q	12.6	13q	45.8	1q	33.8	17q	37.2	2p	33.3	19p	28	7p	46.7	19q	7.5	9p	21.7	5q	50	8q	21.9
7p	10.4	16q	37.5	7q	12	8q	33.3	8q	28.5	6p	34.9	17p	21	19q	21.5	17q	40	1p	5.7	13q	21.7	7p	50	3q	18.8
5p	9.4	8p	25	9p	11.8	12p	33.3	20p	20.3	20q	21	11q	9.3	20q	18.5	20q	40	1q	5.7	Xq	21.7	7q	50	19p	18.8
6p	9.4	8q	25	11q	11.8	17q	29.2	7p	20	2q	16.3	12q	17.8	20p	12.9	8p	33.3	5q	5.7	17q	17.4	17q	50	19q	18.8
13q	9.4	1q	12.5	12q	11.4	2q	25	17q	19.8	3q	14	1q	21.7	1p	10.8	5q	26.7	16q	5.7	Xp	17.4	4p	45	3p	12.5
10p	8.3	2p	12.5	4p	8.4	8p	25	19q	18.9	7p	14	18q	19.4	11q	8.9	9q	26.7	2p	1.9	1q	13	18q	40	1q	9.4
20q	8.3	2q	12.5	5p	8.4	7q	20.8	20q	15.7	20p	14	13q	14	14q	8	15q	26.7	3q	1.9	2p	13	4q	35	1p	6.3
12q	7.3	4p	12.5	6q	8.4	17p	20.8	11q	13.1	7q	11.6	2q	13.2	Xq	7.7	20p	26.7	5p	1.9	2q	13	12q	35	8p	6.3

CR = Chromosomal region; C mel = Cutaneous melanoma; Endo = Endometrial; H&N = Head and neck; MCC = Merkel cell carcinoma; Neuro = Neuroblastoma; Rhab = Rhabdomyosarcoma; GC = Gastric carcinoma; U mel = Uveal melanoma; OSCC = Oral squamous cell carcinoma; GISTS = Gastrointestinal stromal tumours; GE = Gastro-oesophageal adenocarcinoma; Para = Parathyroid adenomas; Pit = Non-functioning pituitary; N endo = Sporadic neuroendocrine tumours of the digestive system. *No distinction was made between oesophageal adenocarcinoma from Barrett's mucosa and gastric adenoacarcinoma arising at the fundus of the stomach. As a result, CGH data for GC and GE could not be merged.

Table 3 The number of altered chromosomal arms observed among the different tumour types

Cancer type	Gains/tumour		Losses/tumour		Total instability (loss + gain) per tumour
Gastric carcinoma	365\68	5.4	128\68	1.9	7.3
Gastrointestinal stromal	52\32	1.6	71\32	2.2	3.8
Head and neck	588\47	12.5	245\47	5.2	17.7
Pancreatic	231\51	4.5	188\51	3.7	8.2
Colorectal	204\80	2.6	190\80	2.4	5
Prostate	312\100	3.1	447\100	4.5	7.6
Testicular	337\41	8.2	171\41	4.2	12.4
Breast	752\187	4	549\187	2.9	6.9
Ovarian	1136\203	5.6	499\203	2.5	8.1
Endometrial	186\33	5.6	50\33	1.5	7.1
Cervical	163\30	5.4	124\30	4.1	9.5
Cutaneous melanoma	203\67	3	227\67	3.4	6.4
Merkel cell carcinoma	23\3	8	13\3	4.3	12.3
Uveal melanoma	23\11	2.1	27\11	2.5	4.6
Renal	346\151	2.3	530\151	3.5	5.8
Bladder	222\96	2.3	278\96	2.9	5.2
Wilms'	89\54	1.6	71\54	1.3	2.9
Connective tissue sarcoma	530\193	2.7	154\193	0.8	3.5
Rhabdomyosarcoma	158\24	6.6	61\24	2.5	9.1
Lung	845\142	6	599\142	4.2	10.2
Liver	201\43	4.7	322\43	7.5	12.2
Neuroblastoma	56\118	0.5	439\118	3.7	4.2
Brain	1152\325	3.5	1076\325	3.3	6.8
Gastro-oesophageal	100\15	6.7	50\15	3.3	10
Parathyroid	38\53	7.2	121\53	2.3	9.5
Pituitary	92\23	4	22\53	4.2	8.2
Neuroendocrine*	162\20	8.1	57\20	2.9	11

*Sporadic neuroendocrine tumours of the digestive system.

those with deficient repair ability (Table 4). As expected, the tumours lacking repair function had a higher frequency of instability. For example, gain of 7p and 7q was seen in 33% of tumours with non-functioning repair genes, while these aberrations were absent in tumours with intact DNA repair phenotype. Although a relationship between genomic instability and both tumour progression and repair deficiency had been previously suggested, CGH has provided strong data to support this hypothesis in tumour specimens.

Ovary

Several studies have been published assessing the genomes of ovarian cancer cases. The available data were split into ovarian cancers derived from the epithelia and those derived from germ cells. Cancers of the epithelia were then further subdivided into sporadic and hereditary cases. The hereditary cases were defined as such based on BRCA1 and BRCA2 status. It is appreciated that some papers did not assess their cases for BRCA1 and BRCA2 and that a small percentage of the sporadic cases may have altered BRCA genes. Overall, however, this division of ovarian tumours has yielded some useful observations. Firstly, it was found that the frequency of genetic aberrations was greatest in the sporadic cases at 41/47 chromosomal arms, compared with 33/47 in hereditary cases and 30/47 in the germ cell tumours. The greatest level of concordance was at 1q and 8q where gains occurred at approximately 30% and 50%, respectively, in all three tumour types. Both hereditary and sporadic cases had a high degree of gain at 3q (40.6% in sporadic and 50% in inherited cases). This is in contrast to the same region being gained in only 5.3% of germ cell tumours. However, all three tumour subtypes are likely to have some common genetic origin based on the observation that regions such as 1q and 8q are gained to an equal extent in all ovarian cancer types so far studied by CGH.

Prostate

The data on prostate cancer allowed comparison of CGH results in patient cohorts with primary resected carcinomas or tumours that recurred after hormone therapy. It has been speculated that further genetic damage allows a subclone of tumour cells to acquire resistance to chemotherapy and such studies can test this hypothesis. Very little change in the frequency of genetic aberration between primary carcinoma and recurrent carcinoma was seen (39/47 in primary vs 42/47 in recurrent). However, differences were seen in the degree of genetic aberration when specific chromosomal regions were considered. For example, gain in chromosome 8q was seen in 25.9% primary carcinomas compared with 73.9% in recurrent cases. Similarly, 19p was lost in 3.7% of primary tumours and 34.8% in recurrent cases. Gain in the region containing the androgen receptor gene, Xp, increased from 7.4% in primary tumour to 28.3% in patients with recurrent disease. This is consistent with androgen receptor gene amplification as a mechanism of resistance to hormone therapy. However, this was not always the case with some regions of the genome only slightly changed in the degree of the aberration between primary and recurrent. For example, 3p was lost in 1.9% of primary tumours and 4.3% in recurrent cases. Generally, the data support the hypothesis that increased tumour aggression is the phenotype of a more unstable genome.

Table 4 Patterns of loss(−) and gain in specific tumour subtypes shown as the percentage of tumours with involvement for selected chromosomes

Tumour type	Colon†						
CR	Iga *n*=14	Hga *n*=12	Carcinoma *n*=16	Min– *n*=6	Min+ *n*=12	Primary *n*=10	Metastases *n*=10
7p	7.1	33.3	50	0	33.3	10	10
7q	0	25	31.3	0	33.3	30	30
8p	0	0	0	0	0	10&–30	10&–80
12q	0	8.3	6.3	0	0	20	0
13q	0	8.3	50	–16.7	41.7	30&–10	50
18q	0	–16.7	–37.5	0	–25	–50	–90
20q	0	33.3	75	0	25	50	40
Involved arms	3\47	21\47	32\47	3\47	22\47	34\47	35\47

Tumour type	Ovary		
CR	Sporadic* *n*=148	Inherited *n*=20	OGCT *n*=19
1q	34.8&–0.7	30	31.6
2q	18.1&–1.4	50&–5	0
3q	40.6	50	5.3&–5.3
8q	52.9&–0.7	55	42.1
21q	5.8&–7.2	0	47.4
Involved arms	41\47	33\47	30\47

Tumour type	Prostate	
CR	Primary *n*=54	Recurrent *n*=46
3p	–1.9	4.3&–4.3
7p	3.7	34.8&–2.2
7q	13&–1.9	34.8&–2.2
8p	–46.5	8.7&–60.9
8q	25.9	73.9
19p	7.4&–3.7	–34.8
Xp	7.4&–1.9	28.3&–8.7
Xq	14.8	15.2&–6.5
Involved arms	39\47	42\47

Tumour type	Sarcoma					
CR	Osteosarcoma *n*=14	RMS-E *n*=10	RMS-A *n*=14	Liposarcoma *n*=14	ASPS *n*=13	Ewing's *n*=20
2p	0	50	50	0	0	5
6p	28.6	0	7.1	0	0	10
2q	7.1	60	–14.3	14.3	0	5
13q	14.3	60&–10	35.7&–7.1	7.1&–21.4	0	5
16q	0	2&–30	7.1&–7.1	7.1	–7.7	5&–5
Involved arms	19\47	38\47	35\47	38\47	14\47	28\47

In several tumour subtypes both loss and gain were observed on the same chromosomal arm. Variation in the number of chromosomal arms involved in genetic instability was also observed between subtypes. *Contains tumours which were not evaluated for BRCA1 and BRCA2 status. † represents data from three separate studies evaluating tumour progression, microsatellite instability and metastasis respectively. CR = chromosomal region; Iga = low-grade adenoma; Hga = high-grade adenoma; OGCT = ovarian germ cell tumours; MIN+ = without microsatellite instability; MIN– = with microsatellite instability; RMS-E = rhabdomyosarcoma embryonal; RMS-A = rhabdomyosarcoma alveolar; ASPS = alveolar soft part sarcoma

Connective tissue tumours

CGH data were available for several tumour types (liposarcoma, alveolar soft part sarcoma, osteosarcoma, Ewing's, rhabdomyosarcoma and osteochondroma). Unlike the other subtypes discussed (colon, ovary and prostate), tumours of the connective tissue are found in many different sites throughout the body. Considering the frequency of genetic aberration, the widest range of variation between subtypes among any tumour type in the literature is observed in the sarcomas. At one end of the spectrum a study on osteochondromas reports no genetic aberrations in 15 cases of this benign tumour type (Larramendy et al, 1997). Such a paper is unique in the CGH literature as all other investigations report some genomic change detectable by CGH. The alveolar soft part- and osteosarcomas show low to moderate frequency of genetic aberration at 14 and 19 out of 47 chromosomal arms respectively. While the other subtypes showed moderate to high numbers of arms involved (range 28–38 of 47). Another unique observation in the CGH literature was seen in an osteosarcoma study where only gain of genetic material was detected (Forus et al, 1995). Caution must be exercised when interpreting such results as it is unlikely that this cancer is the exception where no loss of genetic material is required for its development. More likely any loss, such as that of a tumour suppressor gene, is below detection by CGH. Rhabdomyosarcomas are further subdivided histologically into alveolar and embryonal types. Generally, a higher degree of gain and loss is seen in the embryonal rhabdomyosarcoma compared

with alveolar rhabdomyosarcoma (Weber-Hall et al, 1996). For example, a sub-chromosomal region of 13q is gained in 60% and lost in 10% of embryoneal, while the same region is gained in 35.7% and lost in 7.1% of alveolar, rhabdomyosarcomas. The exception is 2p, which is lost in 50% of cases in both subtypes. Comparing both subtypes of rhabdomyosarcoma with other sarcomas it is observed that a gain of 2q is not present in a high proportion in all sarcomas. In fact no change in 2q is detected in liposarcoma or alveolar soft part sarcoma and gain in Ewing's sarcoma is detected in less than 10% of all cases. This pattern of a certain chromosomal region commonly occurring in a specific subtype, but not in any other, continues for many chromosomal regions, suggesting that sarcomas are very distinct in terms of their genetic origin, with each subtype having its own marker chromosomal aberrations. This may be due to the variation in tissue type in which these tumours arise. No single chromosomal aberration was found to be present in a high proportion of all sarcomas.

PATTERNS OF GENOMIC IMBALANCE OR INSTABILITY IN SOLID TUMOURS

The degree of genomic imbalance detectable by CGH differs significantly between the various solid tumours (Table 3). Chromosomal gain varied from 0.5 to 12.5 chromosome arms per tumour with a median of 4.5, while loss varied from 0.8 to 7.5 chromosomal arms per tumour with a median of 3.3. Total instability (chromosomal loss + chromosomal gain per number of tumours) was highest in head and neck tumours (17.7 lesions per tumour) and testicular (12.4 lesions per tumour) and lowest in Wilms' (2.9 lesions per tumour) and sarcoma (3.5 lesions per tumour) tumours. These frequencies represent an overall value for each specific tumour type, as information on the chromosomal alterations found within an individual tumour was not available in most literature reports of CGH in human solid tumours. Difference in the degree of loss or gain was also observed between the various solid tumours (Table 3). For example, chromosomal gain was observed more frequently than loss in the sarcomas and endometrial tumours, while loss was more frequently observed for renal and liver tumours. It is unknown whether these patterns represent coincidental changes from generalized genomic instability or suggest that some cancers are more likely to be influenced by the loss of tumour suppressor genes (genomic loss), while others are more frequently influenced by oncogene over expression (genomic gain). In addition, several studies have identified an association between the acquisition of genetic aberrations and patient survival (Iwabuchi et al, 1995; Tanner et al, 1995). However, there are discrepancies in this association found in Table 3, and any correlations between biological markers and patient survival need to be interpreted cautiously in the context of modern therapy.

COMPARISON WITH SOLID TUMOUR KARYOTYPE ANALYSIS

Classical karyotyping of metaphase chromosomes has been successfully performed for some solid tumours. A recent review reported the frequencies and distribution of chromosomal imbalances detected in 3185 solid tumours from 11 tumour types using chromosomal banding (Merkel et al, 1997). Overall, deletions were more common than gains in this analysis. Our review has found the opposite, with gains more commonly detected by CGH than losses. This difference may reflect the difficulties with using tumour karyotyping to identify the chromosomal changes that have occurred in tumours with highly complex rearrangements and will be influenced to some extent by amplified segments being hidden among unidentified marker chromosomes. CGH should be more sensitive for the detection of the presence of gains than losses and therefore the discrepancies with the above study are likely to reflect technical limitations of the two methods. By restricting analysis to common alterations (i.e. the gain or loss was detected in at least 15% of the tumours studied for that particular tumour type), the classical karyotyping studies described fewer regions of gain and loss than CGH for every tumour type evaluated. CGH appeared to identify the same alterations described using the karyotyping approach (with the exception of balanced translocations which are not detectable by CGH), but also observed additional regions of loss or gain. For example, only two regions of gain were detected in ovarian carcinoma by traditional cytogenetic analysis compared with 26 regions of gain seen by CGH. However, there have been too few studies of solid tumour cytogenetics using both CGH and chromosome banding for any firm conclusions regarding concordance between the two techniques. Nevertheless, the accumulating body of evidence in the literature suggests that CGH is more sensitive than other current technologies available for global assessment of loss and/or gain in solid tumour genomes.

CONCLUSION

From this review, it is apparent that no specific chromosomal imbalances are found in all cancers, with the most frequently identified regions of gain or loss occurring in 27.7% and 16.3% of tumours respectively. This reflects the heterogeneity in genomic alterations identified in different tumour types. In addition, much variation within tumour subtypes was observed.

The development of CGH has provided the technology to identify many new areas of genomic alteration which were not previously recognized to be altered in tumorigenesis. This has now expanded the number of areas of the genome for which more detailed molecular study is required to give a clearer more complete understanding of cancer biology.

Other areas where CGH could potentially make a significant contribution include its application in tumour diagnosis, as a prognostic tool, or for investigations into chemoresistance. The ability to assess the entire genome in a single experiment makes this technique potentially useful as an adjunct to routine histopathology. Several studies have established the feasibility of using CGH to detect genomic regions involved in the acquisition of resistance in human cancer cell lines and have detected novel regions of the genome not previously recognized to be involved in drug resistance (du Manoir et al, 1997; Wasenius et al, 1997; Leyland-Jones et al, 1998; Rooney et al, 1998). This provides the impetus to apply CGH to human tumour specimens in the context of modern drug therapy to assess its role in optimizing patient treatment.

ACKNOWLEDGEMENTS

Many thanks to Dr Lars-Peter Erwig for translation of a German paper. This work was supported in part by a University of Aberdeen Research Consortium studentship, a University of Aberdeen Equipment Award and an Aberdeen Royal Infirmary endowment grant.

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APPENDIX

Bladder

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