

Chapter 6

Copy Number Changes in Carcinomas: Applications

Henry Wood and Pamela Rabbitts

Contents

6.1 Introduction.....	96
6.2 Relationship Between Oncogenes and Chromosomal Amplifications.....	96
6.3 Chromosomal Deletions and Tumour Suppressor Genes.....	98
6.4 Identification of Functionally Important Cancer Genes.....	99
6.5 Copy Number Changes Associated with Disease Outcome.....	100
6.6 Genome-Wide Assessment of Copy Number Changes.....	101
References.....	101

Abstract The applications of information on copy number changes in cancer have been twofold. Recognizing that regions of copy number gain signalled the location of oncogenes and that, similarly, copy number loss signalled the location of tumour suppressor genes, has resulted in screening of the minimally defined regions for candidate genes involved in tumourigenesis. Once candidates emerged, other evidence of their role in tumours was sought, by functional assays for example, and a huge literature built up describing these gene classes. Even without knowledge of how the genes acted in the development of tumours, the second application has been to correlate the chromosomal abnormalities with various clinical parameters, again resulting in many thousands of publications, although to date the translation of laboratory observations into clinical practice is still not widespread.

Keywords Amplification • Deletion • Oncogene • Tumour suppressor gene • Clinical outcome

H. Wood • P. Rabbitts (✉)

Wellcome Trust Brenner Building, Leeds Institute of Cancer and Pathology, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK
e-mail: P.Rabbitts@leeds.ac.uk

6.1 Introduction

Early studies of tumour karyotypes used direct observation of chromosomes and many examples of abnormalities were observed. Two technical advances moved the field forward: firstly, the development of culture media to grow cancer cells in vitro and, secondly, the discovery that colchicine arrests cells in metaphase making their chromosomes visible using microscopy. When possible, cells growing in short-term cultures were passaged until they became established as immortalized cell-lines and these remain a valuable tool for molecular cell biology. When staining techniques were developed and each individual chromosome could be distinguished [1], tumour-specific chromosomal abnormalities could be enumerated. This enabled the construction of large catalogues such as the *Catalog of Chromosome Aberrations in Cancer* [2] now online but originally a hard-back book. Most of the catalog was devoted to haematological malignancies due to the relative ease of obtaining chromosome spreads from bone marrow or peripheral blood specimens. Solid tumours were often represented by samples from metastatic lesions or effusions and, thus, only typical of late stage disease.

The development of molecular genetics, made possible by DNA cloning, completely changed the approach to the study of copy number in tumours (see Chap. 2). It was no longer necessary to be able to observe chromosomes directly: instead, by measuring the relative proportions of DNA from different regions of the genome, chromosomal gain and loss could be inferred. This meant that it was possible to analyze relatively large numbers of primary tumour samples from patients although the extensive use of cell lines continued. Rather than whole chromosomes or chromosome arms, the better resolution of newer techniques allowed the emphasis to shift to focal regions since this provided the opportunity to identify which genes were involved. In the 1980s and 1990s, the focus was on understanding the biological consequences of gene loss and gain because it became clear that gene copy number gain was a mechanism of activating oncogenes and that gene copy number loss was a method of inactivating tumour suppressor genes. By the end of the twentieth century, conventional chemotherapy was recognized as failing to deliver the hoped for improvements in survival in the major common malignances: understanding the way in which these genes, collectively cancer genes, initiate and maintain the disease was seen as a new approach to identifying drug targets.

6.2 Relationship Between Oncogenes and Chromosomal Amplifications

The chromosomal abnormalities, homogeneously staining regions and double minutes, have been found exclusively in mammalian tumour cells, particularly in cell lines. Using Southern blotting and fluorescence in situ hybridization (FISH) (see Chap. 2), correlative evidence in mouse adrenal tumours suggested that these structures might be the location of amplified genes [3]. The same techniques were used

to make the link between a region of gene amplification and the location of a known oncogene. Since oncogenes, first found in avian retroviruses were known to have a cellular counterpart [4], a cell line, COLO320, with structural evidence of gene amplification, was screened for over-expression of 12 viral oncogenes: only the homolog of viral *myc* was overexpressed [5]. Neither the mechanisms of amplification of the homolog called cMYC nor the biological consequences of its over-expression were known at that time. However this study, by producing probes by molecular cloning for both filter and in situ hybridization, established a valuable approach to associate oncogenes with regions of gene amplification. To be successful, this trio of complementary methodologies worked best on cell-lines and, therefore, was most frequently applied to those tumours for which a large number of cell-lines had been derived. Lung tumours are a good example. In the 1980s, John Minna and Adi Gazdar had considerable success in developing the conditions required to establish lung tumours in culture and showed that members of the MYC family *MYC*, *MYCL*, and *MYCN* were frequently amplified and expressed [6]. Since not all tumour samples can be converted to cell lines, there was concern that the successful group represented the most aggressive diseases but parallel analyses of cell lines and their cognate primary tumours largely dispelled this concern [7].

Not all amplified regions have the benefit of encompassing a homolog of a viral oncogene to guide identification of the pivotal gene, but those amplicons that occur with high frequency have been the subject of intense scrutiny. A good example of this is the amplified regions on 3q. This is very common in all squamous cell carcinomas sometimes occurring as an extra copy of the chromosome arm, as in cervical tumours [8], but also involving minimal regions such as the focal amplifications seen at 3q26 in squamous cell lung cancers [9]. Even then, the region encompasses a large number of genes: several methods have been used to determine the key gene(s) within the amplicon. Often this starts with an educated guess evolving from a knowledge of the characteristics of genes within the amplified region: in the 3q amplicon *TP63*, *P3CA* and *SOX2* have all been favoured [10]. In a few oesophageal tumours, copy number analysis has pinpointed *SOX2* as the only amplified gene in the amplicon. Functional analysis confirmed its role in tumour proliferation when co-transfection with *FOXE1* or *FGFR2* transformed an immortalized (but non-tumourigenic) bronchial epithelial cell-line [11]. In another study *SOX2* and another 3q26 gene, *PPK1* were shown to cooperate to activate hedgehog signalling in a cell model of squamous cell lung cancer [12]. Taking a computational approach to identify co-operating genes within the amplicon identified a further three genes: *SENP2*, *DCUN1D1* and *DVL3* [13]. Confusingly, increased expression has been associated with increased survival in some lung cancer patients [14] and decreased survival in patients with cervical cancer [15].

The narrative of this research, designed to identify the pivotal genes in this very important amplicon, illustrates a current problem. Although the gene order on chromosomes, their copy number and transcription levels are now well documented by high through-put sequencing and the use of expression microarrays, functional assays to confirm the key gene(s) in an amplicon have not kept pace with structural analysis.

6.3 Chromosomal Deletions and Tumour Suppressor Genes

Investigators using classical cytogenetic techniques were able to identify deletions but it was the application of a genetic and molecular genetic approach to a childhood tumour, retinoblastoma [16] that captured their importance for the development of cancer and identified a new class of genes, later called tumour suppressor genes, characterized by the requirement for inactivation of both alleles to elicit a tumorigenic effect – the ‘two hit mechanism’ [17]. Just as viral oncogenes were of value to pinpoint oncogenes involved in human tumours, for tumour suppressor genes (TSGs), inherited cancer syndromes provided a useful route to identify their chromosomal location [18]. Genetic linkage studies were used first to define the chromosomal locus followed by molecular genetic approaches, such as loss of heterozygosity analysis, LOH (see Chap. 2) to define the region further and identify genes that could be examined for mutations by sequence analysis.

A number of TSGs have been identified using this approach such as *APC* [19], *BRCA1* [20] and *BRCA2* [21]. In some situations, the role of the deletions is to delineate candidate genes; in others, finding that a gene already associated with cancer within a deletion can be a validation of its authenticity. This is true for TP53 which was first isolated as a host protein binding to a tumour viral protein (SV40 large T), but gained its tumour suppressor gene status when it was shown to reside in a frequently deleted region on chromosome 17 in colon tumours [22]. In the same study, the gene was shown to be mutated by sequence analysis. Subsequently, *TP53* has been found to be involved in at least 50 % of human cancers [23], and is the subject of tens of thousands of research articles, yet despite being frequently mutated, it has not yet found its way into routine clinical practice either as a disease marker or a drug target [24].

The success in using deletions to pinpoint TSGs was next applied to cancers with no obvious inherited predisposition, since cytogenetic analysis of chromosomes spreads showed evidence of frequent deletions in solid tumours [1]. This was followed by LOH analysis of samples from much larger patient series hoping to define a minimally deleted region to reduce the number of genes that required scrutiny for the presence of mutations. This was a daunting task [24] especially before the human genome was sequenced and the number and order of genes on chromosomes was known. However, both alleles are inactivated by a homozygous deletion within the region of interest in some tumours, thereby limiting the number of genes to be examined, as the deletion has to be compatible with cell viability. Such deletions were used successfully in the identification of a number of TSG, *p16/CDKN2A* [25] and *PTEN* [26] being notable examples. But not all homozygous deletions harbour *bona fide* TSG [27]. Studies on chromosome 3 exemplify this. Deletions of or within the short arm of chromosome 3 are very common in a range of malignant tumours, especially those of the squamous subtype, and occur very early in the development of these tumours and are even occasionally detected in apparently normal epithelium cells (see Chap. 5). Many studies have scrutinized the genes residing in homozygous deletions in 3p without

identifying genes showing frequent mutation [28]. One possible explanation is that although deletion is responsible for the loss of one allele the remaining allele is inactivated by an epigenetic mechanism such as methylation [29]. Although candidate TSGs on 3p were identified, such as *RASSF1* and *FHIT*, and partly validated, their inactivation in mouse models did not produce robust evidence of their independent tumour suppressor function. Furthermore it is becoming clear that, without the benefit of homozygous deletions, LOH is a clumsy tool for positional cloning strategies [24]. Nonetheless a more recent evaluation by the originator of the “two hit hypothesis”, Alfred Knudson, concedes that TSGs may have a role in tumourigenesis through their partial inactivation, and the concept of haploin sufficiency has now been validated for a number of TSGs [30]. A recent example of what this might mean is a study in renal cell carcinoma which showed that genes involved in LOH adjacent to the *VHL* gene, a TSG with a known role in this cancer, were down-regulated, resulting in a network metabolism signature unique to this cancer [31]. Thus, the “one gene at a time” approach that worked so well in the early phase of TSG discovery may be too simplistic and cooperation between genes may be involved in somatically arising tumours [32].

6.4 Identification of Functionally Important Cancer Genes

The identification of consistent copy number changes, amplifications and deletions, can provide strong circumstantial evidence for the involvement of the delineated genes in tumourigenesis. If genes within the candidate regions are frequently mutated in a tumour-specific manner, this greatly increases the conviction that the gene is directly involved in tumour development. Even in this situation, and certainly when candidates have no recurring mutations, functional assays as mentioned above, are needed to confirm the gene’s status, and also to understand the way in which a mutant protein has a tumourigenic effect. Assays for oncogenes have depended on introducing the suspected genes as a cDNA into untransformed cells and scoring for a tumour-related phenotype, usually involving increased proliferation [33]. Conversely, tumour-suppressing potential is assessed by introducing the suspected gene into tumour cell lines and observing a decrease in tumour-related features, such as migration or colony formation [34]. Assays involving tumour formation in nude mice have also been used [35] although, more recently, genetically engineered mice have been the system of choice to recapitulate gene expression in human tumours more closely as described in Chap. 20 in this book. However, traditionally this has been a time-consuming and expensive method. More recently, using the increased data now available for both genomes and transcriptomes, computational methods to identify pathways or networks and expose driver genes have become prevalent [36, 37]. Additionally, biological screens, such as RNAi, are being developed to replace the single gene approach [38]. A recent review of all these methods is provided by Eifert and Powers [39].

6.5 Copy Number Changes Associated with Disease Outcome

Naturally with so many chromosomal regions and interesting genes associated with cancer, the question that is frequently asked is ‘do the genomic and genetic abnormalities have any clinical significance?’ For these translational studies, a knowledge of the gene function is not required; in fact the correlation of a genetic abnormality and a clinically-related phenotype can be another way in which evidence is accumulated to support the importance of a particular gene. Clinical utility ranges across diagnosis, prognosis and prediction of treatment response, including efficacy and toxicity.

An early success was the association of the *MYCN* gene and neuroblastoma. Following on from the discovery that the *MYC* gene is localized to an amplified region in lung tumours [40], other tumour types with known amplifications were tested with probes to *MYC*. In this way, a gene homologous to *MYC* was found to be amplified in neuroblastoma and called *MYCN* [41]. It was of particular interest because the degree to which *MYCN* was amplified was closely associated with the disease stage, demonstrating its value as a prognostic marker [42].

The greatest success in translating laboratory discoveries into the clinic has been obtained for breast cancer. An early observation was that the *ERBB2* gene, more usually now called *HER2/neu* or just *HER2*, was amplified and over-expressed in breast cancer, and that this indicated a poor prognosis [43]. The development of an antibody to the HER2 protein, a receptor on the cell surface, that was shown to be effective in the treatment of HER2 “positive” breast cancer [44, 45] meant that it was essential to develop robust laboratory tests to identify patients who would benefit from HER2-targeted therapies [46]. These tests rely on FISH to detect gene amplification or immunohistochemistry to detect increased levels of the HER2 protein. As such, these tests are only semi-quantitative and subjective and rely on experienced professionals for their interpretation. There is clearly a place for a test based on direct assessment of the patients’ tumour DNA and high through-put sequencing should provide that, although issues of normal cell contamination and inter-tumour heterogeneity will have their own drawbacks.

Although many thousands of studies of both genes and chromosomal regions have been linked to cancer phenotypes [47, 48] described in a database [49], only a very small number have been developed for use in the clinic, such as *HER2* and *EGFR*. There are a number of reasons for this. With regard to prognosis, recurrence and survival, often the information does not directly impact on clinical management because the number of treatment options is limited and there are other confounding factors involved in their selection. A further complication that is currently receiving attention, is the effect of intra-tumour heterogeneity on the distribution of markers and targets [50]. This heterogeneity could result in biopsies failing to reflect the molecular composition of the whole tumour with obvious consequences for clinical management. It has also been appreciated that the conceptual and statistical framework applied to clinical trials needs to be developed for biomarker studies [51]. This will be particularly important for those biomarkers developed for the prediction of

treatment response, including toxicity, as the biomarker would have immediate clinical application [52].

6.6 Genome-Wide Assessment of Copy Number Changes

When gene expression data are used to inform clinical outcomes, rather than single genes, groups of genes are assessed in a single test [53]. This approach may prove useful for copy number data. Although genome-wide copy number evaluation using comparative genomic hybridization (CGH) has been used extensively to provide copy number read-out across the genome, analysis has not usually been of whole genomes, but rather to pinpoint regions of particular interest (see Chap. 2). However, it is possible to use DNA copy number data obtained using microarrays to define patterns of gain and loss within the genome that have distinct relationships with outcomes. Hicks et al. [54] showed that when the whole genomes of breast cancers were defined by “the number and proximity of genomic alterations” they could be segregated into groups with different overall survival. Consideration of the whole genome may be less vulnerable to inter-tumour heterogeneity since many parameters are being assessed simultaneously. Single gene tests have the problem that they might implicate a candidate driver which in reality is only amplified or deleted in a fraction of the tumour mass. They have the additional problem that the driver for any particular tumour might not be the gene being tested. With whole genome measurements, the real drivers will be more likely to be present at an early stage in the disease, so are detected more frequently. If whole genomes are used to identify candidate genes, then computational methods need to be employed to filter all the potential drivers. These whole genome signatures are usually less reliant on the copy number in any one gene, instead measuring the cumulative effects of multiple regions of the genome, or the entire genome. Although microarray analysis has been the most common method for whole genome copy number measurement in recent years, the advent of next-generation sequencing has seen this monopoly eroded. Campbell et al. first described copy number measurement using next-generation sequencing in 2008 [55]. Since then, it has been shown to be possible to use very low coverage data to produce a similar read-out to the microarray method using diagnostic material at low cost [56] making it suitable for clinical use.

References

1. Caspersson T, Zech L, Johansson C (1970) Differential binding of alkylating fluorochromes in human chromosomes. *Exp Cell Res* 60:315–319
2. Mitelman (ed) (1983) *Catalog of chromosome aberrations in cancer*. Wiley-Liss
3. George D, Powers V (1982) Amplified DNA sequences in Y1 mouse adrenal tumor cells: association with double minutes and localization to a homogeneously staining chromosomal region. *Proc Natl Acad Sci U S A* 79:1597–1601

4. Bishop J (1983) Cellular oncogenes and retroviruses. *Annu Rev Biochem* 52:301–354
5. Alitalo K, Schwab M, Lin C, Varmus H, Bishop J (1983) Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-myc) in malignant neuroendocrine cells from a human colon carcinoma. *Proc Natl Acad Sci U S A* 80:1707–1711
6. Johnson B, Brennan J, Ihde D, Gazdar A (1992) myc family DNA amplification in tumors and tumor cell lines from patients with small-cell lung cancer. *J Natl Cancer Inst Monogr* 13:39–43
7. Johnson B, Makuch R, Simmons A, Gazdar A, Burch D et al (1988) myc family DNA amplification in small cell lung cancer patients' tumors and corresponding cell lines. *Cancer Res* 48:5163–5166
8. Ma Y, Wei S, Lin Y, Lung J, Chang T et al (2000) PIK3CA as an oncogene in cervical cancer. *Oncogene* 19:2739–2744
9. Qian J, Massion P (2008) Role of chromosome 3q amplification in lung cancer. *J Thorac Oncol* 3:212–215
10. McCaughan F, Pole J, Bankier A, Konfortov B, Carroll B et al (2010) Progressive 3q amplification consistently targets SOX2 in preinvasive squamous lung cancer. *Am J Respir Crit Care Med* 182:83–91
11. Bass A, Watanabe H, Mermel C, Yu S, Perner S et al (2009) SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat Genet* 41:1238–1242
12. Justilien V, Walsh M, Ali S, Thompson E, Murray N et al (2014) The PRKCI and SOX2 oncogenes are coamplified and cooperate to activate Hedgehog signaling in lung squamous cell carcinoma. *Cancer Cell* 10:139–151
13. Wang J, Qian J, Hoeksema M, Zou Y, Espinosa A et al (2013) Integrative genomics analysis identifies candidate drivers at 3q26-29 amplicon in squamous cell carcinoma of the lung. *Clin Cancer Res* 19:5580–5590
14. Toschi L, Finocchiaro G, Nguyen T, Skokan M, Giordano L et al (2014) Increased SOX2 gene copy number is associated with FGFR1 and PIK3CA gene gain in non-small cell lung cancer and predicts improved survival in early stage disease. *PLoS ONE* 9:e95303
15. Shen L, Huang X, Xie X, Su J, Yuan J et al (2014) High expression of SOX2 and OCT4 indicates radiation resistance and an independent negative prognosis in cervical squamous cell carcinoma. *J Histochem Cytochem* 62:499–509
16. Hansen M, Cavenee W (1988) Retinoblastoma and the progression of tumor genetics. *Trends Genet* 4:125–128
17. Knudson AG Jr (1971) Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 68:820–823
18. White R (1992) Inherited cancer genes. *Curr Opin Genet Dev* 2:53–57
19. Levy D, Smith K, Beazer-Barclay Y, Hamilton S, Vogelstein et al (1994) Inactivation of both APC alleles in human and mouse tumors. *Cancer Res* 54:5953–5958
20. Smith S, Easton D, Evans D, Ponder B (1992) Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. *Nat Genet* 2:128–131
21. Gudmundsson J, Johannesdottir G, Bergthorsson J, Arason A, Ingvarsson S et al (1995) Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12-q13. *Cancer Res* 55:4830–4832
22. Baker S, Fearon E, Nigro J, Hamilton S, Preisinger A et al (1989) Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 244:217–221
23. Hollstein M, Sidransky D, Vogelstein B, Harris C (1991) p53 mutations in human cancers. *Science* 253:49–53
24. Devilee P, Cleton-Jansen A, Cornelisse C (2001) Ever since Knudson. *Trends Genet* 17:569–573
25. Kamb A, Gruis N, Weaver-Feldhaus J, Liu Q, Harshman K et al (1994) A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 264:436–440

26. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI et al (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275:1943–1947
27. Cox C, Bignell G, Greenman C, Stabenau A, Warren W et al (2005) A survey of homozygous deletions in human cancer genomes. *Proc Natl Acad Sci U S A* 102:4542–4547
28. Kok K, Naylor S, Buys C (1997) Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. *Adv Cancer Res* 71:27–92
29. Burbee D, Forgacs E, Zöchbauer-Müller S, Shivakumar L, Fong K et al (2011) Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. *J Natl Cancer Inst* 93:691–699
30. Berger A, Knudson A, Pandolfi P (2011) A continuum model for tumor suppression. *Nature* 476:163–169
31. Gatto F, Nookaew I, Nielsen J (2014) Chromosome 3p loss of heterozygosity is associated with a unique metabolic network in clear cell renal carcinoma. *Proc Natl Acad Sci U S A* 111:E866–E875
32. Watanabe H, Ma Q, Peng S, Adelmant G, Swain D et al (2014) SOX2 and p63 colocalize at genetic loci in squamous cell carcinomas. *J Clin Invest* 124:1636–1645
33. Booden MA, Ulka AS, Der CJ (2006) Cellular assays of oncogene transformation. In: Cellis JE (ed) *Cell biology a laboratory handbook*, published by Elsevier, pp 345–352
34. Brummelkamp T, Bernards R, Agami R (2002) A system for stable expression of short interfering RNAs in mammalian cells. *Science* 296:550–553
35. Engel AM, Schou G (2006) Assays of tumorigenicity in nude mice. In: Cellis JE (ed) *Cell biology a laboratory handbook*, published by Elsevier, pp 353–357
36. Akavia U, Litvin O, Kim J, Sanchez-Garcia F, Kotliar D et al (2010) An integrated approach to uncover drivers of cancer. *Cell* 143:1005–1017
37. Santarius T, Shipley J, Brewer D, Stratton MR, Cooper C (2010) A census of amplified and overexpressed human cancer genes. *Nat Rev Cancer* 10:59–64
38. Sawey E, Chanrion M, Cai C, Wu G, Zhang J et al (2011) Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by Oncogenomic screening. *Cancer Cell* 19:347–358
39. Eifert C, Powers R (2012) From cancer genomes to oncogenic drivers, tumour dependencies and therapeutic targets. *Nat Rev Cancer* 12:572–578
40. Soucek L, Evan G (2010) The ups and downs of Myc biology. *Curr Opin Genet Dev* 20:91–95
41. Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop J et al (1983) Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* 305:245–248
42. Brodeur G, Seeger R, Schwab M, Varmus H, Bishop J (1984) Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 224:1121–1124
43. Slamon D, Clark G, Wong S, Levin W, Ullrich A et al (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235:177–182
44. Cobleigh M, Vogel C, Tripathy D, Robert N, Scholl S et al (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 17:2639–2648
45. Verma S, Miles D, Gianni L, Krop IE, Welslau M et al (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 367:1783–1791
46. Rakha E, Ellis O (2014) Breast cancer: updated guideline recommendations for HER2 testing. *Nat Rev Clin Oncol* 11:8–9
47. Myllykangas S, Himberg J, Böhlting T, Nagy B, Hollmén J et al (2006) DNA copy number amplification profiling of human neoplasms. *Oncogene* 25:7324–7332

48. Myllykangas S, Böhling T, Knuutila S (2007) Specificity, selection and significance of gene amplifications in cancer. *Semin Cancer Biol* 17:42–55
49. Scheinin I, Myllykangas S, Borze I, Böhling T, Knuutila S et al (2008) CanGEM: mining gene copy number changes in cancer. *Nucleic Acids Res* 36:D830–D835
50. Crockford A, Jamal-Hanjani M, Hicks J, Swanton C (2014) Implications of intratumour heterogeneity for treatment stratification. *Pathology* 232:264–273
51. Poste G (2012) Biospecimens, biomarkers, and burgeoning data: the imperative for more rigorous research standards. *Trends Mol Med* 18:717–722
52. Mehta S, Shelling A, Muthukaruppan A, Lasham A, Blenkiron C et al (2010) Predictive and prognostic molecular markers for cancer medicine. *Ther Adv Med Oncol* 2:2125–2148
53. Chen HY, Yu SL, Chen CH, Chang GC, Chen CY et al (2007) A five-gene signature and clinical outcome in non-small-cell lung cancer. *N Engl J Med* 356:11–20
54. Hicks J, Krasnitz A, Lakshmi B, Navin NE, Riggs M et al (2006) Novel patterns of genome rearrangement and their association with survival in breast cancer. *Genome Res* 16:1465–1479
55. Campbell P, Stephens P, Pleasance E, O’Meara S, Li H et al (2008) Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. *Nat Genet* 40:722–729
56. Wood H, Belvedere O, Conway C, Daly C, Chalkley R et al (2010) Using next generation sequencing for high resolution multiplex analysis of copy number variation from nanogram quantities of DNA from formalin fixed paraffin embedded specimens. *Nucl Acid Res* 38:e151