Review

Atsuka Matsui[a], Tatsuya Ihara[a], Hiraku Suda[a], Hirofumi Mikami[a] and Kentaro Semba*

Gene amplification: mechanisms and involvement in cancer

Abstract: Gene amplification was recognized as a physiological process during the development of *Drosophila melanogaster*. Intriguingly, mammalian cells use this mechanism to overexpress particular genes for survival under stress, such as during exposure to cytotoxic drugs. One well-known example is the amplification of the dihydrofolate reductase gene observed in methotrexate-resistant cells. Four models have been proposed for the generation of amplifications: extrareplication and recombination, the breakage-fusion-bridge cycle, double rolling-circle replication, and replication fork stalling and template switching. Gene amplification is a typical genetic alteration in cancer, and historically many oncogenes have been identified in the amplified regions. In this regard, novel cancer-associated genes may remain to be identified in the amplified regions. Recent comprehensive approaches have further revealed that co-amplified genes also contribute to tumorigenesis in concert with known oncogenes in the same amplicons. Considering that cancer develops through the alteration of multiple genes, gene amplification is an effective acceleration machinery to promote tumorigenesis. Identification of cancer-associated genes could provide novel and effective therapeutic targets.

Keywords: amplicon; cancer; development; drug resistance; microarray.

List of abbreviations: Akt, v-akt murine thymoma viral oncogene homolog; AR, androgen receptor; BCL2, B-cell CLL/lymphoma 2; BFB, breakage-fusion-bridge; bHLHZ, basic-helix-loop-helix zipper; BIR, break-induced replication; BRAF, v-Raf murine sarcoma viral oncogene homolog B; BRCA2, breast cancer 2, early onset; BRD4, bromodomain-containing 4; CAD, carbamoyl-synthetase 2 aspartate transcarbamylase and dihydroorotase; CCND1, cyclin D1; CDK, cyclin-dependent kinase; CGH, comparative genomic hybridization; C-MYC, v-myc avian myelocytomatosis viral oncogene homolog; CNV, copy number variation; CRPC, castration-resistant prostate cancer; DHFR, dihydrofolate reductase; DM, double minute chromosome; DRCR, double rolling-circle replication; DSB, double-stranded break; EGFR, epidermal growth factor receptor; EMSY, chromosome 11 open reading frame 30; ERBB2, v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; ERα, estrogen receptor alpha; FISH, fluorescence *in situ* hybridization; FoSTeS, replication fork stalling and template switching; GRB7, growth factor receptor-bound protein 7; HR, homologous recombination; HSR, homogeneously staining region; MAPK, mitogen-activated protein kinase; MAX, MYC associated factor X; MDM2, mouse double minute 2 homolog; MEK, mitogen-activated protein kinase kinase 1; MITF, microphthalmia-associated transcription factor; MTX, methotrexate; NGS, next-generation sequencing; PAK1, P21-activated kinase 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PLP1, proteolipid protein 1; RARα, retinoic acid receptor α; RAS, rat sarcoma viral oncogene homolog; RB, retinoblastoma; StAR, steroidogenic acute regulatory protein; STARD3, START domain containing 3; START, StAR-related lipid transfer; STAT3, signal transducer and activator of transcription 3; TKI, tyrosine kinase inhibitor; TOP2A, DNA topoisomerase 2-α.

[a]These authors contributed equally to this article.
***Corresponding author: Kentaro Semba,** Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University, 2-2 Wakamatsu-cho, Shinjuku, Tokyo 162-8480, Japan, e-mail: ksemba@waseda.jp
Atsuka Matsui, Tatsuya Ihara, Hiraku Suda and Hirofumi Mikami: Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University, 2-2 Wakamatsu-cho, Shinjuku, Tokyo 162-8480, Japan

Introduction

Gene amplification is defined as an increase in copy number of a restricted region of a chromosome arm (1). This amplified region is called an ‘amplicon’. Gene

amplification plays a crucial role in the normal developmental program, including in the amplification of chorion genes in *Drosophila melanogaster* ovaries (2) and amplification of rDNA in amphibian oocytes (3). Gene amplification in somatic cells was first observed by Breuer and Pavan (1955) during a morphological study of salivary gland chromosomes of *Rhynchosciara americana* larvae, a species of fly (4). The authors found that certain loci on salivary gland chromosomes swelled enormously, producing 'DNA puffs', and that the quantity of DNA in these loci increased compared with other loci, either simultaneously or after the swelling (5). In 1957, Rudkin and Corlette provided the first experimental evidence of gene amplification in *Rhynchosciara angelae* by measuring the quantity of absorbing material in a chromosome using microspectrophotometry (6). Large quantities of these developmental genes encoding rRNAs and structural proteins for eggshells and cocoons are necessary during development. In recent studies, two additional amplicons encoding a variety of proteins, including transporters and proteases, were identified in *Drosophila* follicle cells by a comparative genomic hybridization (CGH) array approach (7). In addition to its functions in physiological processes, gene amplification has attracted much attention for its involvement in cellular adaptation against cytotoxic drugs and, interestingly, in tumorigenesis (1, 8).

The first demonstration of gene amplification in cultured mammalian cells was the amplification of the dihydrofolate reductase (*DHFR*) gene in the AT3000 line of methotrexate (MTX)-resistant murine sarcoma 180 cells in 1978 (9). DHFR catalyzes the reduction of dihydrofolate to tetrahydrofolate, which is used in glycine, purine, and thymidylate synthesis. Methotrexate, a 4-amino analog of folic acid, inhibits DHFR activity and thus causes arrest of DNA replication and cytotoxicity (10). After exposure of cells to methotrexate, the surviving cells exhibited an increased copy number of the *DHFR* gene. This phenomenon was observed in several cell types, including Chinese hamster ovary cells as well as the AT3000 cell line. The occurrence of gene amplification in drug-resistant cells was observed not only with *DHFR* but also with the carbamoyl-synthetase 2 aspartate transcarbamylase and dihydroorotase (*CAD*) gene. Amplification of the *CAD* gene was observed in Syrian hamster cells resistant to *N*-phosphonacetyl-L-aspartate, an inhibitor of aspartate transcarbamylase (11).

The amplification mechanism of the *DHFR* gene in MTX-resistant cells has been frequently employed for recombinant protein production in mammalian cells (12, 13). Because the production of protein pharmaceuticals, including cytokines and humanized antibodies, requires the use of cultured mammalian cells, a robust method for the production of large amounts of recombinant protein in mammalian cells is critically important. In the general method for mammalian cell-mediated production of recombinant proteins, the plasmid harboring the recombinant gene and a second plasmid containing a selection gene are first co-introduced into cells. The *DHFR* gene is the most frequently used selection gene. By adding MTX to the cells, the target gene co-amplifies gradually (13). Although almost all cells die by MTX treatment, the surviving cells that overproduce DHFR frequently contain several hundred to a few thousand copies of the recombinant gene (14). This method allows the production of cells that hyper-produce the target gene. Most *DHFR*-amplified cells produce up to 10- to 20-fold more recombinant protein than unamplified cells (12).

In 1976, Biedler and Spengler analyzed the amplified DNA in the MTX-resistant mammalian cell line and observed elongated chromosomal structures, which they named 'homogeneously staining regions' (HSRs) (15). Trypsin-Giemsa staining was used to visualize the differential banding pattern along the length of metaphase chromosomes, and staining showed that the long chromosome segments in drug-resistant Chinese hamster cells did not show any discrete 'bands' as did normal Chinese hamster cells (15). HSRs were thus easily recognizable.

Double minute chromosomes (DMs), which are small and often paired extrachromosomal elements, were also observed in MTX-resistant Chinese hamster cells (16). DMs vary in size among different cell lines and even within the same cells. They replicate in the cell cycle without centromeres (17). The *DHFR* gene tends to be amplified either on DM or in a HSR configuration, depending on the cell line, although the reason for such tendency remains unclear (17).

In this review, we first discuss the mechanisms of gene amplification and then focus on gene amplification in cancer. We will introduce typical oncogenes localized in amplicons and discuss their therapeutic potential.

Mechanisms of generation of amplicons

As described above, gene amplification is detected in two forms: HSRs and DMs. DMs are circular extrachromosomal elements that replicate autonomously and lack a centromere and telomeres (18). Although gene amplification is often observed in cancer and other degenerative

disorders, the mechanisms of amplicon generation remain largely unknown. Four hypotheses have been proposed to explain the generation of amplicons: extrareplication and recombination, the breakage-fusion-bridge (BFB) cycle, double rolling-circle replication (DRCR), and replication fork stalling and template switching (FoSTeS).

Extrareplication and recombination

In 1972, Smith and Vinograd found that extrachromosomal circles were produced by transient treatment of cultured human cells with cycloheximide (19). Later, Woodcock and Cooper showed that transient inhibition of DNA synthesis by cycloheximide induced aberrant replication in human cultured cells, in which some DNA fragments were replicated more than once in normal replication (20). A previous study of chromosomes showed that all primary neuroblastoma cells had only DMs, although neuroblastoma cell lines cultured for a longer period of time had DMs and/or HSRs (21). These data imply that the primary product of amplification is DMs, and that HSRs are then produced by DMs. Furthermore, Osheim and Miller discovered multiforked chromosomal structures, called 'bubbles', in the follicle cells of *Drosophila*, where the chorion gene was amplified (22). These studies propose a model of extrareplication and recombination shown in Figure 1.

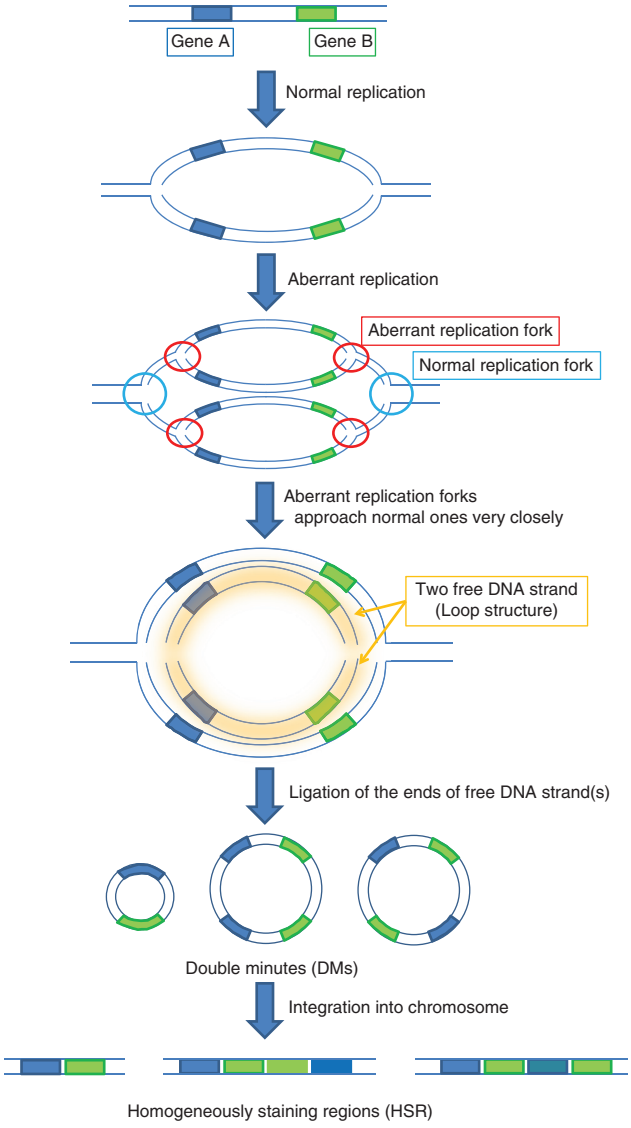


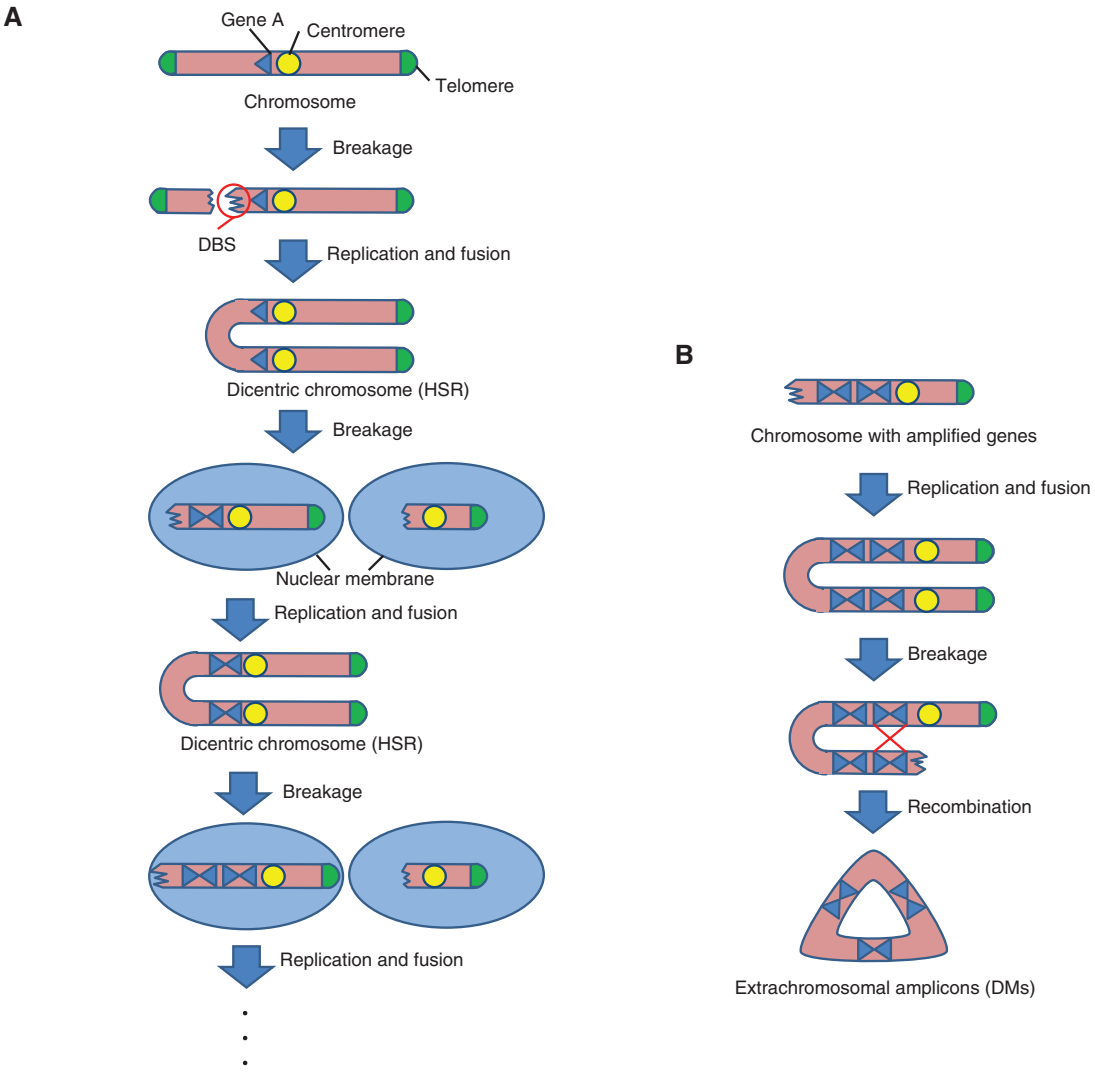
Figure 1 Model of gene amplification generated by extrareplication and recombination (17, 37, 105).
This type of amplification is triggered by aberrant replication in a replication fork, resulting from a single additional initiation of replication within the replication fork. In this replication fork, there is a loop structure including the two free DNA strands (106). These strands can form three types of DMs by ligase. Some of these circular DNAs would be recombined into chromosomes, generating HSRs.

BFB cycle

The BFB cycle model (Figure 2) was proposed by McClintock using *Zea mays* in 1951 (23). She confirmed that BFB cycles occurred in chromosome 9 following X-ray treatment that induced a double-stranded break (DSB). BFB cycles could be induced by some clastogenic drugs in Chinese hamster fibroblast cells, and clones in which gene amplification occurred were resistant to these drugs (24). BFB cycles have also been observed in human cancer cells. For example, four osteosarcoma cell lines showed the existence of anaphase bridges, dicentrics, chromosomal anomalies, and multipolar mitotic figures (25).

DRCR

The DRCR system (Figure 3) was proposed by Futcher in 1986 for amplification of the yeast circular 2 micron plasmid (26), and later experimentally confirmed by Volkert and Broach (27). Subsequently, a similar system was proposed for drug resistance gene amplification (28). In 2005, Watanabe and Horiuchi described a novel DRCR system of gene amplification based on break-induced replications (BIRs) in yeast (29). The authors used engineered yeast, in which a DSB occurs on a chromosome, and it yielded products similar to HSRs and DMs observed in higher eukaryotes. This system was recently improved for yeast and mammalian cells by using the cre-lox system



BFB cycles generates gene amplification with inverted repeats(HSR).

BFB cycles generates extrachromosomal amplicons (DMs).

Figure 2 The BFB cycle.
First, a DSB occurs in a chromosome, such that it loses a telomere (107). By replicating, sister chromatids that lack telomeres are produced. The two ends lacking telomeres fuse and form a dicentric chromosome. At anaphase, the two centromeres in the dicentric chromosome are pulled apart by kinetochore microtubules and form a chromatid bridge. This abnormal separation of the chromosome causes a random position on the chromatid bridge to be broken, and produces a new end lacking a telomere. This end fuses, forming a new dicentric chromosome and produces a new end without a telomere. Repeated cycles generate amplification with inverted repeats (HSR) (A) and extrachromosomal amplicons (DMs) (B) (108).

to induce DRCR (30). The DRCR system also yielded HSR/DM-type products similar to those of cancer cells. Although there is no evidence that DRCR naturally occurs in cancer cells, these findings imply a relationship between DRCR and gene amplification in tumor cells.

FoSTeS

The FoSTeS model (Figure 4) was proposed by Slack, Thornton, Magner, Rosenberg, and Hastings in 2006 to explain gene amplification in *Escherichia coli* under stress (31). The authors discovered three pieces of data supporting FoSTeS. First, the junction sequences between amplified regions showed as little homology as 4–15 bp, indicating that homologous recombination (HR) does not occur, and were separated by 7–32 kb, which is too long for amplification to occur within a replication fork. Second, the 5′ exonuclease domain of DNA polymerase I was essential for amplification, suggesting that the lagging strand is involved in this amplification. Third, the frequency of amplification was reduced by

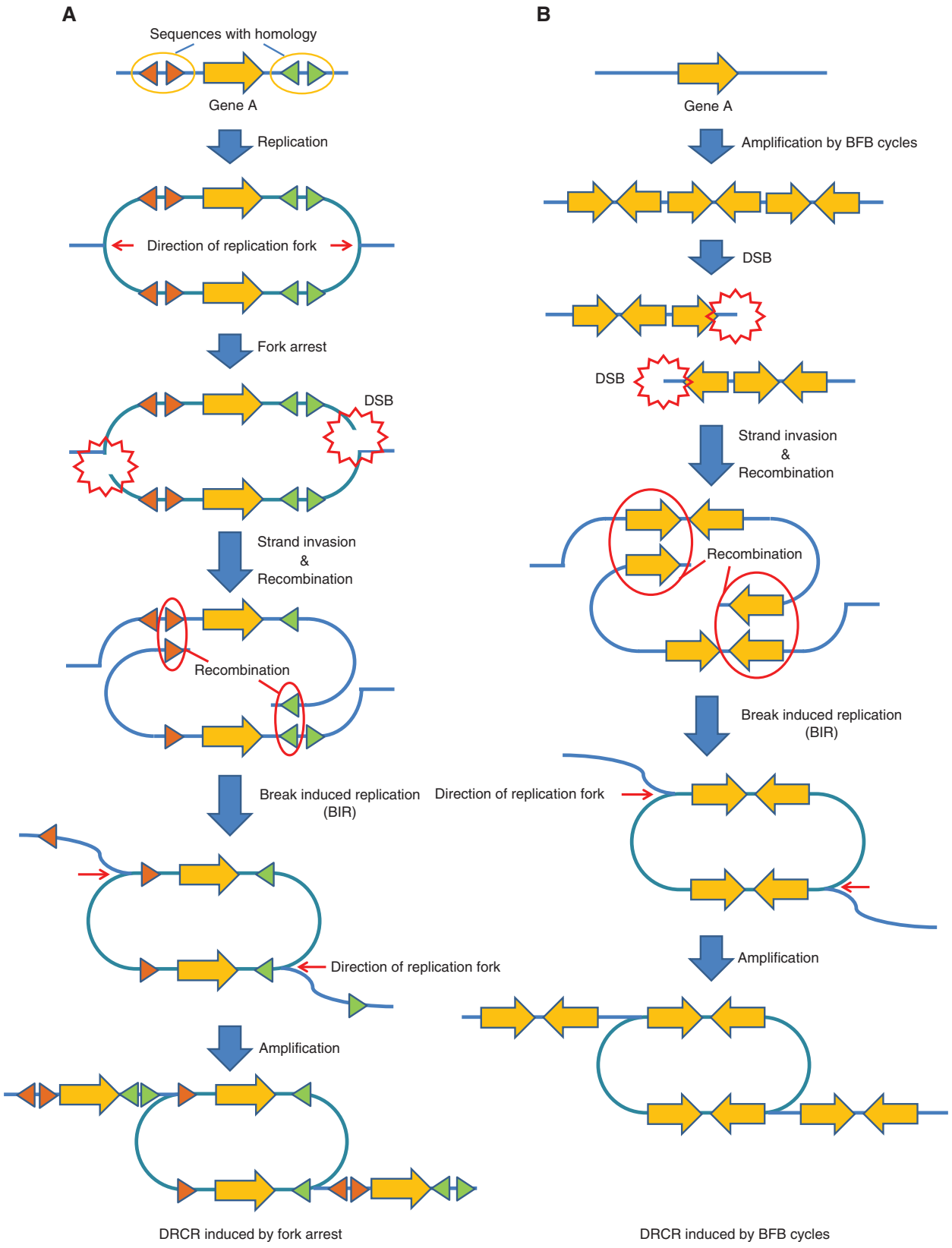


Figure 3 DRCR model of generation of amplicons proposed by Watanabe and Horiuchi (30).
If the replication fork arrests (A) or BFB cycles occur (B) and subsequently cause a DSB on a chromosome, BIRs (109) between sequences with homology occur in both broken ends, and two replication forks chase each other, facilitating gene amplifications.

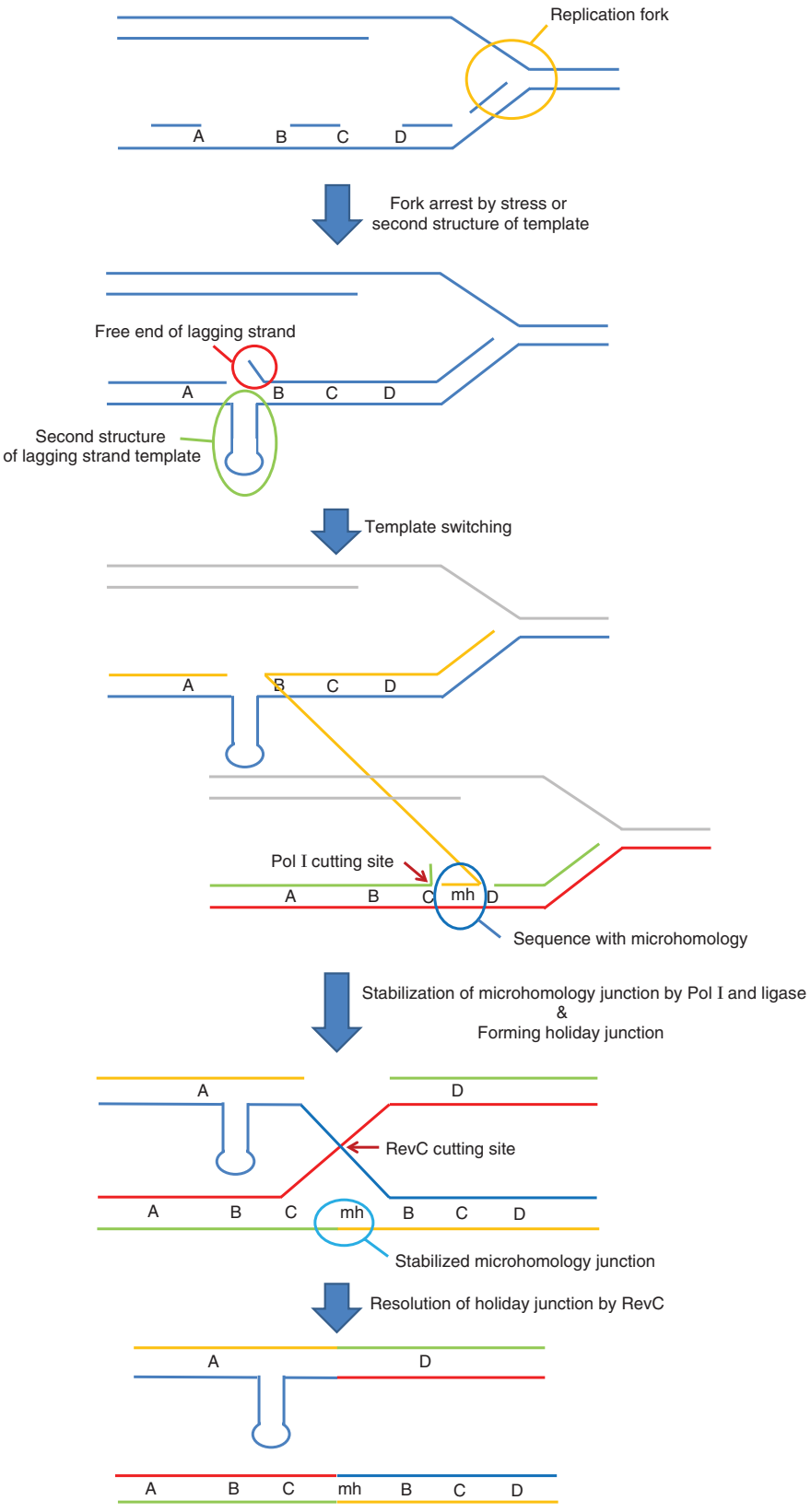


Figure 4 FoSTeS.
When the replication fork stalls, a single-stranded lagging strand is exposed and anneals to the lagging strand template of another replication fork, forming a microhomology junction by polymerase I and ligase. Two products are generated by RuvC-mediated resolution of the Holliday junction.

overexpression of the Exo I 3′ exonuclease, indicating that the 3′ ends of DNA are important for amplification. A previous study suggested that FoSTeS may occur in Pelizaeus-Merzbacher disease patients, in which amplification of the proteolipid protein 1 (*PLP1*) gene was found (32). Furthermore, another study supported the FoSTeS model to explain genomic rearrangement and sequential complexities of two regions on the short arm of human chromosome 17 associated with several diseases: reciprocal Potocki-Lupski microduplication syndrome, Smith-Magenis microdeletion syndrome, reciprocal Charcot-Marie-Tooth disease type 1A, and duplication/hereditary neuropathies with liability to pressure palsies (33).

Gene amplification in cancer

Gene amplification, or genomic DNA copy number aberration, is frequently observed in some solid tumors and has been thought to contribute to tumor evolution (1, 34–36). Chromosomal aberrations are found in DMs, HSRs, or interspersed regions throughout the genome. These occur in a wide variety of tumors and appear to be common in neoplastic cells and very rare in non-neoplastic cells (35, 36). Importantly, amplified oncogenes, such as *MYC*, *MYCN*, epidermal growth factor receptor (*EGFR*), and v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*), have been found in amplicons (34, 37). *MYC* was the first oncogene that was proven to be amplified in a variety of tumor cells, including the neuroendocrine line derived from colon carcinoma, small-cell lung carcinoma, plasma cell leukemia, and murine osteocarcinoma (34, 37).

Gene amplification of oncogenes could be detected by several methods, including DNA-based techniques (PCR or Southern blot), molecular cytogenetic techniques (FISH, fluorescence *in situ* hybridization) with gene-specific probes, and comparative genomic hybridization (CGH) (38). PCR, Southern blot and FISH are mostly restricted to the analysis of known amplified genes (38). In contrast, CGH does not show this limitation. Genome-wide scanning of amplified chromosomal regions with CGH enables the detection of previously unknown amplified genes (38, 39), but it has limited sensitivity and resolution (39, 40). Therefore, amplification and copy number losses and gains can only be detected at limited resolution, in the 5–10 Mbase, and 2 Mbase levels, by standard CGH techniques if metaphase chromosomes are used as the target (39). To overcome this limitation, array-based CGH was developed for both detection and mapping of amplified genes in 1998 (39, 41). The method of using well-defined genomic clones, such as bacterial artificial chromosome (BAC), P1 bacteriophage artificial chromosome (PAC), or cosmid clones is currently widely used (39, 41).

The use of next-generation sequencing (NGS), copy number variation (CNV), and structural variation observed in tumors has been recently extensively analyzed. NGS demonstrated high sensitivity and high-throughput abilities in terms of types and sizes of variants that can be detected (42, 43). Analysis of the cancer genome enables the identification of regions of significant CNVs and novel oncogene candidates as potential therapeutic targets (44, 45). Especially, NGS can be used to identify unknown fusion genes as diagnostic and therapeutic targets. In the following section, typical cancer-associated genes in the amplicons are described in detail.

Oncogenes in the amplicons

One of the most investigated amplicons is localized on chromosome 17. In the 17q12-q21 amplicon, various cancer-associated genes are co-amplified with *ERBB2*, an important ‘driver’ oncogene for breast cancer. Here, we refer to the 17q12-q21 amplicon as the *ERBB2* amplicon. From the clinical perspective, *ERBB2* amplification occurs in 10–34% of breast cancer and this amplification is a significant predictor of relapse and survival in human breast cancer (46–48). In breast cancer, gene amplification of *ERBB2* is well correlated with its protein overexpression. Moreover, *ERBB2* amplification is observed also in gastric and esophageal cancers (49–51). When *ERBB2* is amplified, heterodimer formation between ERBB2 and other EGFR family members on the cell surface is promoted. Formation of the heterodimer is considered to activate proliferation and survival signaling pathways, and thus contribute to oncogenesis (52). *ERBB2* amplification may cause the formation of not only heterodimers but also homodimers, and this may also contribute to oncogenesis. Thus, ERBB2 has been a target of anticancer agents. Trastuzumab, a 95% humanized monoclonal antibody, is one of the well-known anticancer agents against ERBB2 that targets the extracellular domain of the ERBB2 receptor. This drug is effective in breast cancer patients with tumors exhibiting high expression or amplification of *ERBB2*.

Recent studies demonstrate that multiple overexpressed genes in amplicons, in addition to the typical driver oncogenes, also contribute to tumorigenesis. In

fact, several genes that are co-amplified with *ERBB2* are involved in tumorigenesis and progression. GRB7 (growth factor receptor-bound protein 7) is known to bind receptor tyrosine kinases and mediate signals to downstream effectors (53). Moderate expression of ERBB2 alone did not generate transformed foci; however, co-expression of GRB7 together with ERBB2 efficiently induced foci (54). Moreover, GRB7 was reported to bind ERBB2 with high affinity (55). Together, these results support the oncogenic cooperation between ERBB2 and GRB7.

Retinoic acid receptor alpha (RARα) is also co-amplified with *ERBB2* in 23–32% of human *ERBB2*-positive breast cancers. The function of RARα in ERBB2-positive tumors is somewhat controversial. One report shows that RARα is associated with sensitivity to the antiproliferative/cytodifferentiating action of all-*trans* retinoic acid, which stabilizes the effect of lapatinib, a dual inhibitor of EGFR and ERBB2 (56, 57).

Steroidogenic acute regulatory protein (StAR)-related lipid transfer domain containing 3 (STARD3), also known as metastatic lymph node 64 protein (MLN64), in the *ERBB2* amplicon is a protein that independently contributes to the malignant transformation of cancer. Via the StAR-related lipid transfer (START) domain, STARD3 binds cholesterol and promotes steroidogenesis. Overexpression of STARD3 thus promotes steroid hormone production in cancer cells, which may support the growth of hormone-responsive tumors (58, 59).

DNA topoisomerase 2-alpha (*TOP2A*) shows amplification independent of *ERBB2*, despite its vicinity to *ERBB2* on the chromosome, suggesting that the region at the *ERBB2* locus is composed of at least two distinct amplicons that harbor either *ERBB2* or *TOP2A* (49, 56, 60, 61). However, more cases with *TOP2A* aberrations were reported for tumors with ERBB2 amplification than those without amplification (56). The TOP2A enzyme generates DNA breaks during cell division, and thus is essential for the cell cycle (62). TOP2A is targeted by cytotoxic agents such as anthracyclines, etoposide, and teniposide (52). In breast cancer, *TOP2A* amplification is a predictive marker for response to anthracyclines (63). Together these studies show that the significance of gene amplification is to simultaneously increase the amount of factors that are involved in tumorigenesis or progression either independently or cooperatively.

In addition to chromosome 17, amplicons also exist in other chromosomes (Figure 5) (64). Microphthalmia-associated transcription factor (*MITF*), located on 3p13, is a lineage-specific oncogene that is occasionally amplified in melanoma (65). MITF targets genes involved in cell-cycle arrest (*p21* and *INK4a*), cell proliferation (*TBX2* and *CDK2*), and cell survival (B-cell CLL/lymphoma 2, *BCL2*) and differentiation (*TYR*, *TYRP1*, *DCT*, *MART1*, *AIM-1*, and *PMEL17*) (66). *MITF* is amplified in approximately 10–20% of primary melanoma cases, and the incidence is higher in metastatic melanoma. Furthermore, in metastatic melanoma, *MITF* amplification is related to a decrease of five years in survival (65). In addition, MITF can be used as an epithelioid melanoma marker, but not as a marker for rarer desmoplastic and spindle-cell melanoma variants (67, 68).

EGFR gene amplification in chromosome 7p12 causes many types of cancers, including malignant melanoma, breast cancer, colorectal cancer, and lung cancer (64, 69). EGFR is a member of the receptor tyrosine kinase ErbB family that is involved in cell proliferation, apoptosis resistance, survival, invasion, and migration. Ligand binding to EGFR induces a conformational change of the receptor and subsequent homo- or heterodimerization with other ErbB family proteins that result in the autophosphorylation of a tyrosine residue near the C-terminus. Downstream signaling pathways, such as RAS-RAF mitogen-activated protein kinase (MAPK) or PI3K-Akt pathways, are then activated via the phosphorylated tyrosine residues of EGFR (70). In glioblastoma multiforme, which accounts for the majority of malignant gliomas, EGFR overexpression is correlated with poor overall survival (71). *EGFR* amplification and overexpression are predictors of EGFR-targeted drugs.

One of the most successful EGFR-targeted therapies is the class of small molecule tyrosine kinase inhibitors (TKIs), which include gefitinib and erlotinib. TKIs block EGFR-mediated signal transduction by competing with ATP for binding to the tyrosine kinase domain of EGFR (72). In non-small-cell lung cancer, *EGFR* amplification is correlated with better response to gefitinib, which is more effective for patients carrying a somatic mutation in the *EGFR* gene (64). Conversely, some aberrations, like *MET* amplification, were reported to confer TKI resistance to EGFR-related cancer (73, 74). Therefore, combination therapies that target both EGFR and secondary aberrations would be more practical.

Another therapy is the EGFR-specific monoclonal antibody cetuximab. Unlike TKIs, cetuximab prevents EGFR signaling transduction by interfering with ligand binding, resulting in inhibition of EGFR dimerization and autophosphorylation. In addition, cetuximab reportedly induces EGFR internalization and destruction (72). Although cetuximab was proven to be efficient for EGFR in colorectal cancer, the presence of a *KRAS* mutation is significantly associated with poor response (75). Other studies demonstrate that v-Raf murine sarcoma viral

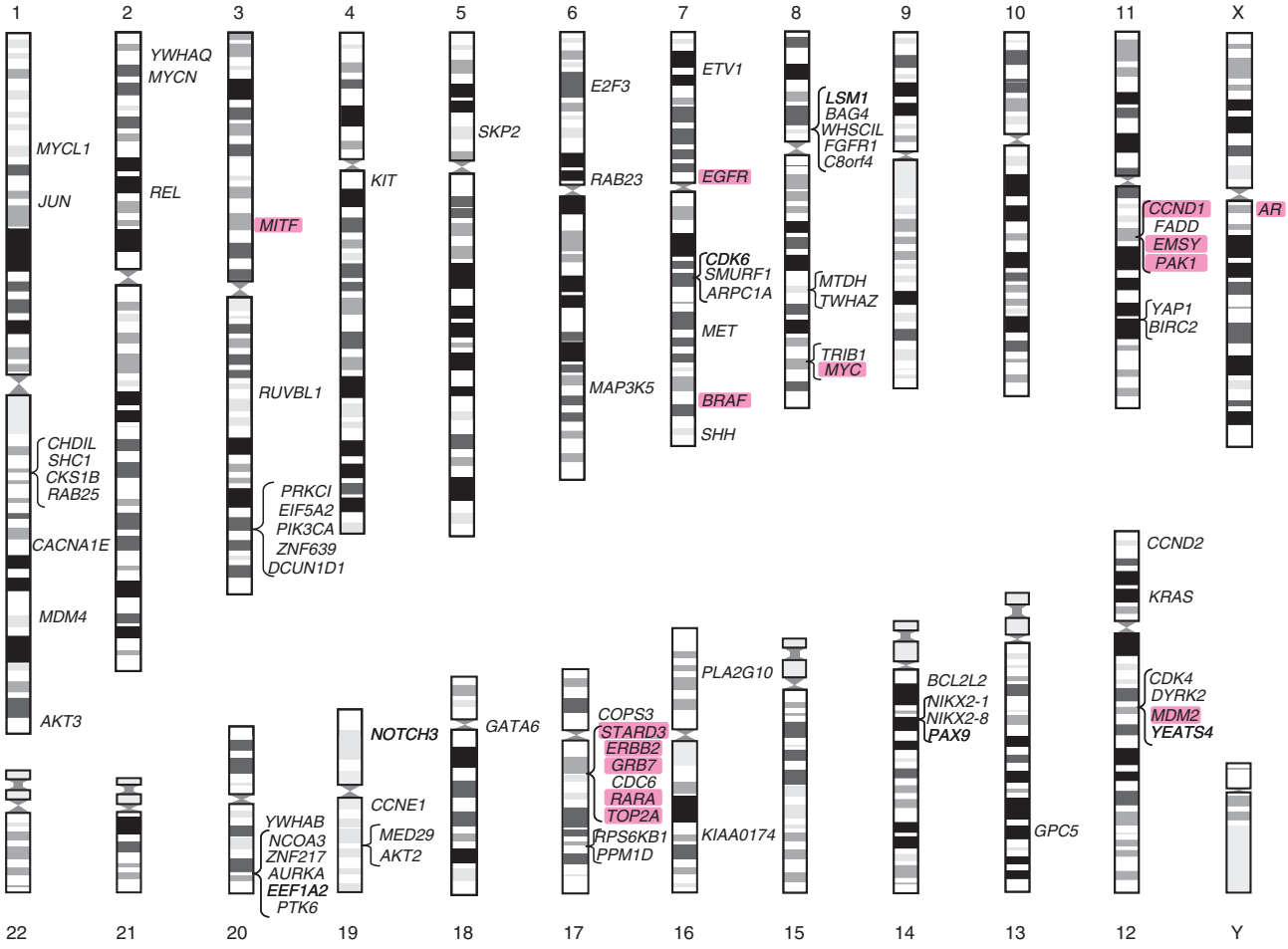


Figure 5 Amplicons on various chromosomes.
Genes mentioned in this review are highlighted.

oncogene homolog B (*BRAF*) mutation and *PI3KCA* mutation are also related to cetuximab resistance (76, 77). It may be necessary to examine not only *EGFR* amplification or mutation, but also the status of other drug resistance-related genes.

Mouse double minute 2 homolog (*MDM2*) gene amplification in chromosome 12q14 is found in at least 19 types of tumors, including sarcoma, esophageal carcinoma, and neuroblastoma (78). MDM2 is a RING domain protein that plays an oncogenic role by inhibiting the p53 tumor suppressor. In most tumors, p53 is frequently inactivated by point mutation or deletion. However, even in cases in which *TP53* status is not aberrant, MDM2 can abrogate the transcriptional activity of p53, which results in the increase of tumorigenic potential. In fact, transgenic mice that contain multiple copies of *Mdm2* transgene exhibit 100% incidence of tumorigenesis (79). Because cancers caused by *MDM2* amplification do not require the deregulation of p53, *MDM2* amplification and *TP53* aberration tend to be mutually exclusive. Namely, *MDM2* amplification functions as a surrogate for loss or mutation of p53. Because of its critical role in tumor incidence, MDM2 could be a therapeutic target. Through chemical library screening, Nutlin 3a was identified as a small-molecule MDM2 antagonist (80). Nutlin 3a competes with p53 for binding to the MDM2 N-terminus, and therefore disrupts the p53-MDM2 interaction, which results in the recovery of wild-type p53. The Nutlin 3a derivative RG7112 is currently in clinical trials (81).

The cyclin D1 (*CCND1*) gene on chromosome 11q13 that encodes the cyclin D1 protein is amplified in various cancers, including breast, head and neck, lung, and bladder cancers (82). CCND1 forms a complex with cyclin-dependent kinase (CDK) 4 or 6 and phosphorylates retinoblastoma (RB) protein, thereby initiating the G1/S cell-cycle transition (83). Furthermore, CCND1 has a CDK-independent role as a transcriptional regulator. CCND1

interacts with various steroid hormone receptors and transcription factors, including estrogen receptor-alpha (ERα), androgen receptor (AR), peroxisome proliferator activated receptor gamma (PPARγ), and signal transducer and activator of transcription 3 (STAT3) (84). In breast cancer, *CCND1* amplification is found in 15–20% of tumors (64, 84). Most *CCND1* amplifications are observed in ER-positive breast cancer and associated with poor prognosis and resistance to tamoxifen therapy (85).

CCND1 amplification is frequently accompanied by co-amplification of the chromosome 11 open reading frame 30 (*EMSY*) gene, which is located at the same locus as *CCND1* 11q13. EMSY binds to the transactivation (TA) domain of breast cancer 2, early onset (BRCA2) and consequently inhibits its transcriptional activity (86). Therefore, *EMSY* amplification can be a substitute for loss or mutation of BRCA2 in breast cancer. Not only is *EMSY* amplification associated with poor prognosis, but patients with co-amplification of *EMSY* and *CCND1* also show worse outcomes than those with neither or one of the *CCND1* and *EMSY* genes amplified (85). P21 activated kinase 1 (*PAK1*), located at 11q13, also shows occasional co-amplification with *CCND1* (87). PAK1 phosphorylates ERα, resulting in induction of upregulation of ERα-controlled proteins, like CCND1 (88). Therefore, PAK1 predicts for tamoxifen resistance.

Because *CCND1* amplification is one of the most common copy number alterations in human cancer (44), inhibiting CCND1 function could be an attractive therapy. As the well-known role of CCND1 is to induce the G1/S transition together with CDK4/6, CDK inhibitors that compete with ATP for binding to the active site of the kinase have been developed, and some have progressed to clinical trials (83). However, although such drugs exhibit beneficial effects to some extent, therapies focused on inhibiting CDK activity are incomplete, as CCND1 has CDK-independent functions. To repress the entire negative response caused by CCND1, therapies that regulate CCND1 at the protein level would be required.

The v-myc avian myelocytomatosis viral oncogene homolog (*C-MYC*) amplification in chromosome 8q24 is one of the most frequent copy number alterations in human cancer (44). C-MYC is a basic-helix-loop-helix-zipper (bHLHZ) transcription factor that activates various genes related to cell proliferation, apoptosis and inhibition of differentiation. C-MYC exerts its effect by forming a heterodimer with its partner bHLHZ protein MYC associated factor X (MAX), followed by binding specific DNA sequences called E-box sequences (89). In breast, colon, and lung cancers, *C-MYC* is proposed as the driver gene at the 8q24 amplicon (1). In addition, patients carrying *C-MYC* amplification have a two-fold increase in risk of relapse and death (90). Because C-MYC induces apoptosis, a synergistic action with anti-apoptotic mechanisms, such as overexpression of BCL2 or BCL-X_L or loss of the tumor suppressor p53, has been observed (89).

Owing to the broad range of C-MYC expression in human cancer, C-MYC has the potential to be a therapeutic target. Indeed, a study using the dominant-negative MYC mutant Omomyc in a transgenic mouse model demonstrated the feasibility of MYC-targeted therapy (91). Although developing a therapy that directly targets C-MYC is considered to be difficult, two studies have proven that the small-molecule bromodomain inhibitor JQ1 exhibits an anti-cancerous effect in multiple myeloma and acute myeloid leukemia, in which C-MYC is essential for tumor maintenance, both *in vitro* and *in vivo* (92, 93). Bromodomain-containing 4 (BRD4) binds to the acetylated lysine residue on histone proteins, resulting in transcriptional activation of cell-cycle-related genes. Bromodomain inhibition by JQ1 confers a repression of C-MYC-induced transcription and, surprisingly, MYC transcription itself. JQ1 has the potential to be a useful therapy for other cancers associated with C-MYC.

Gene amplification and drug resistance

AR gene amplification in chromosome Xq11-13 is found in castration-resistant prostate cancers (CRPCs) (94). AR is a steroid hormone receptor that regulates cell growth and survival. Androgen binding causes a conformational change of AR that leads to AR-ligand complex translocation to the nucleus and subsequent gene transcription (95). Because all prostate cancers except for rare small cell carcinomas express AR at both the mRNA and protein levels, androgen ablation therapy has been the standard treatment (94). However, many prostate cancers are found to acquire resistance during the therapy, and such CRPCs have been reported to carry *AR* gene aberration. *AR* amplification is one of the genetic changes that occur in CRPCs. Approximately 80% of CRPCs exhibit increased *AR* gene copy numbers, and approximately 30% have high levels of amplification (94). *AR* amplification is correlated with increased expression, resulting in the increase of sensitivity to androgen (95). Interestingly, *AR* amplification was not observed before androgen ablation therapy in clinical tumors, suggesting that

AR amplifying cells that can proliferate under low levels of circulating androgen were selected during the therapy (96). Although CRPCs can respond to low androgen levels, their proliferation and survival are still androgen-dependent. Accordingly, combined therapy with gonadotropin-releasing hormone analogs and anti-androgens might be effective (97).

BRAF gene amplification in chromosome 7q34 also confers drug resistance. *BRAF* is a proto-oncogene that is mutated in several cancers, including melanomas and colorectal cancers (98). BRAF protein is a regulator kinase of the MAPK pathway. The most frequent mutation in BRAF, V600E, predicts for sensitivity to AZ628, an inhibitor of BRAF and to CI-1040 and its derivative PD0325901, both of which are inhibitors of mitogen-activated protein kinase kinase 1 (MEK), a kinase in the MAPK pathway downstream of BRAF (99, 100). Conversely, increased activity of CRAF which belongs to the same family as BRAF and a point mutation in MEK1 was identified as the mechanism that provides the resistance for AZ628 and the MEK inhibitor AZD6244 (selumetinib), respectively (101, 102). Recently, a colorectal cancer cell line model showed that V600E *BRAF* amplification also confers resistance to AZD6244 (103). *BRAF* amplification has been found in clinical colorectal cancer tumors. *BRAF* amplification is also found in 20% of malignant melanoma cell lines, although no significant correlation between copy-number increase and BRAF expression was observed (104). In colorectal cancer cell lines, V600E *BRAF* amplification increased the abundance of phosphorylated MEK and hindered the ability of MEK inhibitors (103). Interestingly, cells not treated with BRAF inhibitors included a small population of *BRAF* amplified cells (103). Therefore, clonal selection of cells carrying *BRAF* amplification might have occurred as a result of prolonged exposure to the drug. Because simultaneous inhibition of BRAF and MEK restores the sensitivity of BRAF inhibitor-resistant cells (103), a combined therapy has the potential to be an effective approach.

Expert opinion and outlook

In this review, we have introduced the model of gene amplification and showed several examples of amplicons in cancer. A considerable number of oncogenes have been identified in amplicons and their functions in cancer have been analyzed in detail, which emphasizes the significance of gene amplification during tumorigenesis. Furthermore, molecular targeted therapies such as trastuzumab have been developed to inhibit the functions of amplified oncogenes. Thus, it is reasonable to search for novel cancer-associated genes in amplicons if gene amplification accelerates the processes of tumorigenesis and progression. One typical approach is to knock down each gene to evaluate its function. We are also trying to express all the amplified genes simultaneously in a cell, however, novel experimental techniques need to be developed for this purpose. Even though several models to explain the mechanisms of gene amplification have been proposed so far, the complete mechanisms remain to be resolved. Understanding of the mechanisms may lead to the development of diagnostic markers as well as novel therapeutic approaches against gene amplification.

Highlights

- Gene amplification was first discovered in the developmental program of *Drosophila melanogaster* and thereafter recognized in the response to cytotoxic drugs. Gene amplification is also observed in cancer and is one of the major causes of tumorigenesis.
- There are four models for the mechanisms of generation of amplicons: extrareplication and recombination, BFB cycle, DRCR and FoSTeS.
- Oncogenes are often observed in amplicons in cancer, and thus analyses have been conducted to identify novel oncogenes in amplicons. Several co-amplified genes also contribute to tumorigenesis in concert with oncogenes. Amplified oncogenes have the potential to serve as diagnostic and therapeutic targets.

Acknowledgments: We thank all members of Semba laboratory for encouraging us to write this article. Especially we thank Lynn Yoshimatsu for her critical reading of this manusicript and giving valuable comments.

Received August 12, 2013; accepted September 2, 2013

References

1. Albertson DG. Gene amplification in cancer. Trends Genet 2006; 22: 447–55.
2. Spradling AC, Mahowald AP. Amplification of genes for chorion proteins during oogenesis in Drosophila melanogaster. Proc Natl Acad Sci USA 1980; 77: 1096–100.
3. Brown DD, Dawid IB. Specific gene amplification in oocytes. Oocyte nuclei contain extrachromosomal replicas of the genes for ribosomal RNA. Science 1968; 160: 272–80.
4. Lara FJ. Gene amplification in Rhynchosciara (1955-1987). Memórias Do Instituto Oswaldo Cruz 1987; 82 (Suppl 3): 125–8.
5. Breuer ME, Pavan C. Behavior of polytene chromosomes of Rhynchosciara angelae at different stages of larval development. Chromosoma 1955; 7: 371–86.
6. Rudkin GT, Corlette SL. Disproportionate synthesis of DNA in a polytene chromosome region. Proc Natl Acad Sci USA 1957; 43: 964–8.
7. Claycomb JM, Orr-Weaver TL. Developmental gene amplification: insights into DNA replication and gene expression. Trends Genet 2005; 21: 149–62.
8. Savelyeva L, Schwab M. Amplification of oncogenes revisited: from expression profiling to clinical application. Cancer Lett 2001; 167: 115–23.
9. Alt FW, Kellems RE, Bertino JR, Schimke RT. Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells. J Biol Chem 1978; 253: 1357–70.
10. Schimke RT, Kaufman RJ, Alt FW, Kellems RF. Gene amplification and drug resistance in cultured murine cells. Science 1978; 202: 1051–5.
11. Wahl GM, Padgett RA, Stark GR. Gene amplification causes overproduction of the first three enzymes of UMP synthesis in N-(phosphonacetyl)-L-aspartate-resistant hamster cells. J Biol Chem 1979; 254: 8679–89.
12. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol 2004; 22: 1393–8.
13. Pallavicini MG, DeTeresa PS, Rosette C, Gray JW, Wurm FM. Effects of methotrexate on transfected DNA stability in mammalian cells. Mol Cell Biol 1990; 10: 401–4.
14. Wurm FM, Gwinn KA, Kingston RE. Inducible overproduction of the mouse c-myc protein in mammalian cells. Proc Natl Acad Sci USA 1986; 83: 5414–8.
15. Biedler JL, Spengler BA. Metaphase chromosome anomaly: association with drug resistance and cell-specific products. Science 1976; 191: 185–7.
16. Kaufman RJ, Brown PC, Schimke RT. Amplified dihydrofolate reductase genes in unstably methotrexate-resistant cells are associated with double minute chromosomes. Proc Natl Acad Sci USA 1979; 76: 5669–73.
17. Schimke RT. Gene amplification in cultured animal cells. Cell 1984; 37: 705–13.
18. Storlazzi CT, Lonoce A, Guastadisegni MC, Trombetta D, D'Addabbo P, Daniele G, L'Abbate A, Macchia G, Surace C, Kok K, Ullmann R, Purgato S, Palumbo O, Carella M, Ambros PF, Rocchi M. Gene amplification as double minutes or homogeneously staining regions in solid tumors: origin and structure. Genome Res 2010; 20: 1198–206.
19. Smith CA, Vinograd J. Small polydisperse circular DNA of HeLa cells. J Mol Biol 1972; 69: 163–78.
20. Woodcock DM, Cooper IA. Evidence for double replication of chromosomal DNA segments as a general consequence of DNA replication inhibition. Cancer Res 1981; 41: 2483–90.
21. Brodeur GM, Green AA, Hayes FA, Williams KJ, Williams DL, Tsiatis AA. Cytogenetic features of human neuroblastomas and cell lines. Cancer Res 1981; 41: 4678–86.
22. Osheim YN, Miller OL. Novel amplification and transcriptional activity of chorion genes in Drosophila melanogaster follicle cells. Cell 1983; 33: 543–53.
23. McClintock B. Chromosome organization and genic expression. Cold Spring Harb Symp Quant Biol 1951; 16: 13–47.
24. Coquelle A, Pipiras E, Toledo F, Buttin G, Debatisse M. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. Cell 1997; 89: 215–25.
25. Selvarajah S, Yoshimoto M, Park PC, Maire G, Paderova J, Bayani J, Lim G, Al-Romaih K, Squire JA, Zielenska M. The breakage-fusion-bridge (BFB) cycle as a mechanism for generating genetic heterogeneity in osteosarcoma. Chromosoma 2006; 115: 459–67.
26. Futcher AB. Copy number amplification of the 2 micron circle plasmid of Saccharomyces cerevisiae. J Theor Biol 1986; 119: 197–204.
27. Volkert FC, Broach JR. Site-specific recombination promotes plasmid amplification in yeast. Cell 1986; 46: 541–50.
28. Hyrien O, Debatisse M, Buttin G, de Saint Vincent BR. The multicopy appearance of a large inverted duplication and the sequence at the inversion joint suggest a new model for gene amplification. EMBO J 1988; 7: 407–17.
29. Watanabe T, Horiuchi T. A novel gene amplification system in yeast based on double rolling-circle replication. EMBO J 2005; 24: 190–8.
30. Watanabe T, Tanabe H, Horiuchi T. Gene amplification system based on double rolling-circle replication as a model for oncogene-type amplification. Nucleic Acids Res 2011; 39: e106.
31. Slack A, Thornton PC, Magner DB, Rosenberg SM, Hastings PJ. On the mechanism of gene amplification induced under stress in Escherichia coli. PLoS Genet 2006; 2: e48.
32. Lee JA, Carvalho CMB, Lupski JR. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell 2007; 131: 1235–47.
33. Zhang F, Khajavi M, Connolly AM, Towne CF, Batish SD, Lupski JR. The DNA replication FoSTeS/MMBIR mechanism can generate genomic, genic and exonic complex rearrangements in humans. Nat Genet 2009; 41: 849–53.
34. Klein G, Klein E. Conditioned tumorigenicity of activated oncogenes. Cancer Res 1986; 46: 3211–24.
35. Pall ML. Gene-amplification model of carcinogenesis. Proc Natl Acad Sci USA 1981; 78: 2465–8.
36. Albertson DG, Collins C, McCormick F, Gray JW. Chromosome aberrations in solid tumors. Nat Genet 2003; 34: 369–76.
37. Schwab M, Amler LC. Amplification of cellular oncogenes: a predictor of clinical outcome in human cancer. Genes Chromosomes Cancer 1990; 1: 181–93.
38. Heiskanen MA, Bittner ML, Chen Y, Khan J, Adler KE, Trent JM, Meltzer PS. Detection of gene amplification by genomic hybridization to cDNA microarrays. Cancer Res 2000; 60: 799–802.

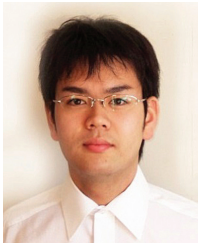
39. Inazawa J, Inoue J, Imoto I. Comparative genomic hybridization (CGH)-arrays pave the way for identification of novel cancer-related genes. Cancer Sci 2004; 95: 559–63.
40. Piper J, Rutovitz D, Sudar D, Kallioniemi A, Kallioniemi OP, Waldman FM, Gray JW, Pinkel D. Computer image analysis of comparative genomic hybridization. Cytometry 1995; 19: 10–26.
41. Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 1998; 20: 207–11.
42. Campbell PJ, Stephens PJ, Pleasance ED, O'Meara S, Li H, Santarius T, Stebbings LA, Leroy C, Edkins S, Hardy C, Teague JW, Menzies A, Goodhead I, Turner DJ, Clee CM, Quail MA, Cox A, Brown C, Durbin R, Hurles ME, Edwards PAW, Bignell GR, Stratton MR, Futreal PA. Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. Nat Genet 2008; 40: 722–9.
43. Koboldt DC, Larson DE, Chen K, Ding L, Wilson RK. Massively parallel sequencing approaches for characterization of structural variation. Methods Mol Biol 2012; 838: 369–84.
44. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, Mc Henry KT, Pinchback RM, Ligon AH, Cho Y-J, Haery L, Greulich H, Reich M, Winckler W, Lawrence MS, Weir BA, Tanaka KE, Chiang DY, Bass AJ, Loo A, Hoffman C, Prensner J, Liefeld T, Gao Q, Yecies D, Signoretti S, Maher E, Kaye FJ, Sasaki H, Tepper JE, Fletcher JA, Tabernero J, Baselga J, Tsao M-S, Demichelis F, Rubin MA, Janne PA, Daly MJ, Nucera C, Levine RL, Ebert BL, Gabriel S, Rustgi AK, Antonescu CR, Ladanyi M, Letai A, Garraway LA, Loda M, Beer DG, True LD, Okamoto A, Pomeroy SL, Singer S, Golub TR, Lander ES, Getz G, Sellers WR, Meyerson M. The landscape of somatic copy-number alteration across human cancers. Nature 2010; 463: 899–905.
45. Kawazu M, Ueno T, Kontani K, Ogita Y, Ando M, Fukumura K, Yamato A, Soda M, Takeuchi K, Miki Y, Yamaguchi H, Yasuda T, Naoe T, Yamashita Y, Katada T, Choi YL, Mano H. Transforming mutations of RAC guanosine triphosphatases in human cancers. Proc Natl Acad Sci USA 2013; 110: 3029–34.
46. Kauraniemi P, Kallioniemi A. Activation of multiple cancer-associated genes at the ERBB2 amplicon in breast cancer. Endocr Relat Cancer 2006; 13: 39–49.
47. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177–82.
48. Yamamoto T, Saito M, Kumazawa K, Doi A, Matsui A, Takebe S, Amari T, Oyama M, Semba K. ErbB2/HER2: its contribution to basic cancer biology and the development of molecular targeted therapy. In: Gunduz M, Gunduz E, editors. Breast cancer – carcinogenesis, cell growth and signalling pathways. Croatia: InTech, 2011: 139–70.
49. Olsen KE, Knudsen H, Rasmussen BB, Balslev E, Knoop A, Ejlertsen B, Nielsen KV, Schönau A, Overgaard J. Amplification of HER2 and TOP2A and deletion of TOP2A genes in breast cancer investigated by new FISH probes. Acta Oncol 2004;43: 35–42.
50. Yano T, Doi T, Ohtsu A, Boku N, Hashizume K, Nakanishi M, Ochiai A. Comparison of HER2 gene amplification assessed by fluorescence in situ hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer. Oncol Rep 2006; 15: 65–71.
51. Mimura K, Kono K, Hanawa M, Mitsui F, Sugai H, Miyagawa N, Ooi A, Fujii H. Frequencies of HER-2/neu expression and gene amplification in patients with oesophageal squamous cell carcinoma. Br J Cancer 2005; 92: 1253–60.
52. Järvinen TAH, Liu ET. HER-2/neu and topoisomerase IIα in breast cancer. Breast Cancer Res Treat 2003; 78: 299–311.
53. Pero SC, Daly RJ, Krag DN. Grb7-based molecular therapeutics in cancer. Expert Rev Mol Med 2003; 5: 1–11.
54. Saito M, Kato Y, Ito E, Fujimoto J, Ishikawa K, Doi A, Kumazawa K, Matsui A, Takebe S, Ishida T, Azuma S, Mochizuki H, Kawamura Y, Yanagisawa Y, Honma R, Imai J, Ohbayashi H, Goshima N, Semba K, Watanabe S. Expression screening of 17q12-21 amplicon reveals GRB7 as an ERBB2-dependent oncogene. FEBS Lett 2012; 586: 1708–14.
55. Stein D, Wu J, Fuqua SA, Roonprapunt C, Yajnik V, D'Eustachio P, Moskow JJ, Buchberg AM, Osborne CK, Margolis B. The SH2 domain protein GRB-7 is co-amplified, overexpressed and in a tight complex with HER2 in breast cancer. EMBO J 1994; 13: 1331–40.
56. Mano MS, Rosa DD, De Azambuja E, Ismael GFV, Durbecq V. The 17q12-q21 amplicon: Her2 and topoisomerase-IIα and their importance to the biology of solid tumours. Cancer Treat Rev 2007; 33: 64–77.
57. Paroni G, Fratelli M, Gardini G, Bassano C, Flora M, Zanetti A, Guarnaccia V, Ubezio P, Centritto F, Terao M, Garattini E. Synergistic antitumor activity of lapatinib and retinoids on a novel subtype of breast cancer with coamplification of ERBB2 and RARA. Oncogene 2012; 31: 3431–43.
58. Watari H, Arakane F, Moog-Lutz C, Kallen CB, Tomasetto C, Gerton GL, Rio MC, Baker ME, Strauss JF. MLN64 contains a domain with homology to the steroidogenic acute regulatory protein (StAR) that stimulates steroidogenesis. Proc Natl Acad Sci USA 1997; 94: 8462–7.
59. Tsujishita Y, Hurley JH. Structure and lipid transport mechanism of a StAR-related domain. Nat Struct Biol 2000; 7: 408–14.
60. Järvinen TA, Tanner M, Bärlund M, Borg A, Isola J. Characterization of topoisomerase IIα gene amplification and deletion in breast cancer. Genes Chromosomes Cancer 1999; 26: 142–50.
61. Hicks DG, Yoder BJ, Pettay J, Swain E, Tarr S, Hartke M, Skacel M, Crowe JP, Budd GT, Tubbs RR. The incidence of topoisomerase IIα genomic alterations in adenocarcinoma of the breast and their relationship to human epidermal growth factor receptor-2 gene amplification: a fluorescence *in situ* hybridization study. Hum Pathol 2005; 36: 348–56.
62. Lee S, Jung S-R, Heo K, Byl JAW, Deweese JE, Osheroff N, Hohng S. DNA cleavage and opening reactions of human topoisomerase IIα are regulated via Mg^{2+}-mediated dynamic bending of gate-DNA. Proc Natl Acad Sci USA 2012; 109: 2925–30.
63. Glynn RW, Miller N, Kerin MJ. 17q12-21-the pursuit of targeted therapy in breast cancer. Cancer Treat Rev 2010; 36: 224–9.
64. Santarius T, Shipley J, Brewer D, Stratton MR, Cooper CS. A census of amplified and overexpressed human cancer genes. Nat Rev Cancer 2010; 10: 59–64.
65. Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, Beroukhim R, Milner DA, Granter SR, Du J, Lee C, Wagner SN, Li C, Golub TR, Rimm DL, Meyerson ML, Fisher DE, Sellers WR. Integrative genomic analyses identify MITF as

a lineage survival oncogene amplified in malignant melanoma. Nature 2005; 436: 117–22.
66. Levy C, Khaled M, Fisher DE. MITF: master regulator of melanocyte development and melanoma oncogene. Trends Mol Med 2006; 12: 406–14.
67. Granter SR, Weilbaecher KN, Quigley C, Fletcher CD, Fisher DE. Microphthalmia transcription factor: not a sensitive or specific marker for the diagnosis of desmoplastic melanoma and spindle cell (non-desmoplastic) melanoma. Am J Dermatopathol 2001; 23: 185–9.
68. Granter SR, Weilbaecher KN, Quigley C, Fisher DE. Role for microphthalmia transcription factor in the diagnosis of metastatic malignant melanoma. Appl Immunohistochem Mol Morphol 2002; 10: 47–51.
69. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Cavenee WK. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 2007; 21: 2683–710.
70. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 2006; 12: 5268–72.
71. Shinojima N, Tada K, Shiraishi S, Kamiryo T, Kochi M, Nakamura H, Makino K, Saya H, Hirano H, Kuratsu J-I, Oka K, Ishimaru Y, Ushio Y. Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. Cancer Res 2003; 63: 6962–70.
72. Taylor TE, Furnari FB, Cavenee WK. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. Curr Cancer Drug Targets 2012; 12: 197–209.
73. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale C-M, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Jänne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007; 316: 1039–43.
74. Pao W, Wang TY, Riely GJ, Miller VA, Pan Q, Ladanyi M, Zakowski MF, Heelan RT, Kris MG, Varmus HE. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2005; 2: e17.
75. Cappuzzo F, Varella-Garcia M, Finocchiaro G, Skokan M, Gajapathy S, Carnaghi C, Rimassa L, Rossi E, Ligorio C, Di Tommaso L, Holmes AJ, Toschi L, Tallini G, Destro A, Roncalli M, Santoro A, Jänne PA. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. Br J Cancer 2008; 99: 83–9.
76. Di Nicolantonio F, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 2008; 26: 5705–12.
77. Sartore-Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 2009; 69: 1851–7.
78. Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. Nucleic Acids Res 1998; 26: 3453–9.
79. Jones SN, Hancock AR, Vogel H, Donehower LA, Bradley A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. Proc Natl Acad Sci USA 1998; 95: 15608–12.
80. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004; 303: 844–8.
81. Wade M, Li Y-C, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer 2013; 13: 83–96.
82. Schwab M. Amplification of oncogenes in human cancer cells. Bioessays 1998; 20: 473–9.
83. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. Nat Rev Cancer 2011; 11: 558–72.
84. Gautschi O, Ratschiller D, Gugger M, Betticher DC, Heighway J. Cyclin D1 in non-small cell lung cancer: a key driver of malignant transformation. Lung Cancer 2007; 55: 1–14.
85. Brown LA, Johnson K, Leung S, Bismar TA, Benítez J, Foulkes WD, Huntsman DG. Co-amplification of CCND1 and EMSY is associated with an adverse outcome in ER-positive tamoxifen-treated breast cancers. Breast Cancer Res Treat 2010; 121: 347–54.
86. Hughes-Davies L, Huntsman D, Ruas M, Fuks F, Bye J, Chin S-F, Milner J, Brown LA, Hsu F, Gilks B, Nielsen T, Schulzer M, Chia S, Ragaz J, Cahn A, Linger L, Ozdag H, Cattaneo E, Jordanova ES, Schuuring E, Yu DS, Venkitaraman A, Ponder B, Doherty A, Aparicio S, Bentley D, Theillet C, Ponting CP, Caldas C, Kouzarides T. EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. Cell 2003; 115: 523–35.
87. Bostner J, Ahnström Waltersson M, Fornander T, Skoog L, Nordenskjöld B, Stål O. Amplification of CCND1 and PAK1 as predictors of recurrence and tamoxifen resistance in postmenopausal breast cancer. Oncogene 2007; 26: 6997–7005.
88. Balasenthil S, Barnes CJ, Rayala SK, Kumar R. Estrogen receptor activation at serine 305 is sufficient to upregulate cyclin D1 in breast cancer cells. FEBS Lett 2004; 567: 243–7.
89. Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. Nat Rev Cancer 2002; 2: 764–76.
90. Deming SL, Nass SJ, Dickson RB, Trock BJ. C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. Br J Cancer 2000; 83: 1688–95.
91. Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, Sodir NM, Karnezis AN, Swigart LB, Nasi S, Evan GI. Modelling Myc inhibition as a cancer therapy. Nature 2008; 455: 679–83.
92. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011; 146: 904–17.
93. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 2011; 478: 524–8.
94. Waltering KK, Urbanucci A, Visakorpi T. Androgen receptor (AR) aberrations in castration-resistant prostate cancer. Mol Cell Endocrinol 2012; 360: 38–43.
95. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001; 1: 34–45.
96. Koivisto P, Kononen J, Palmberg C, Tammela T, Hyytinen E, Isola J, Trapman J, Cleutjens K, Noordzij A, Visakorpi T, Kallioniemi OP.

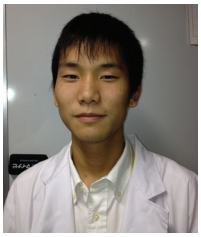
Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res 1997; 57: 314–9.
97. Palmberg C, Koivisto P, Kakkola L, Tammela TL, Kallioniemi OP, Visakorpi T. Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. J Urol 2000; 164: 1992–5.
98. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JWC, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature 2002; 417: 949–54.
99. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N. BRAF mutation predicts sensitivity to MEK inhibition. Nature 2006; 439: 358–62.
100. McDermott U, Sharma SV, Dowell L, Greninger P, Montagut C, Lamb J, Archibald H, Raudales R, Tam A, Lee D, Rothenberg SM, Supko JG, Sordella R, Ulkus LE, Iafrate AJ, Maheswaran S, Njauw CN, Tsao H, Drew L, Hanke JH, Ma X-J, Erlander MG, Gray NS, Haber DA, Settleman J. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. Proc Natl Acad Sci USA 2007; 104: 19936–41.
101. Montagut C, Sharma S V, Shioda T, McDermott U, Ulman M, Ulkus LE, Dias-Santagata D, Stubbs H, Lee DY, Singh A, Drew L, Haber DA, Settleman J. Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res 2008; 68: 4853–61.
102. Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, Hatton C, Chopra R, Oberholzer PA, Karpova MB, MacConaill LE, Zhang J, Gray NS, Sellers WR, Dummer R, Garraway LA. MEK1 mutations confer resistance to MEK and B-RAF inhibition. Proc Natl Acad Sci USA 2009; 106: 20411–6.
103. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. Sci Signal 2010; 3: ra84.
104. Tanami H, Imoto I, Hirasawa A, Yuki Y, Sonoda I, Inoue J, Yasui K, Misawa-Furihata A, Kawakami Y, Inazawa J. Involvement of overexpressed wild-type BRAF in the growth of malignant melanoma cell lines. Oncogene 2004;23: 8796–804.
105. Stark GR, Debatisse M, Giulotto E, Wahl GM. Recent progress in understanding mechanisms of mammalian DNA amplification. Cell 1989; 57: 901–8.
106. Vogelstein B, Pardoll DM, Coffey DS. Supercoiled loops and eucaryotic DNA replicaton. Cell 1980; 22: 79–85.
107. Hastings PJ, Lupski JR, Rosenberg SM, Ira G. Mechanisms of change in gene copy number. Nat Rev Genet 2009; 10: 551–64.
108. Tanaka H, Yao M-C. Palindromic gene amplification – an evolutionarily conserved role for DNA inverted repeats in the genome. Nat Rev Cancer 2009; 9: 216–24.
109. Kraus E, Leung WY, Haber JE. Break-induced replication: a review and an example in budding yeast. Proc Natl Acad Sci USA 2001; 98: 8255–62.

Atsuka Matsui is a graduate student in Semba's lab, Waseda University. She received a Bachelor of Science degree from Waseda University in 2011. She is currently working on transcription factors which transform a non-transformed breast epithelial cell line, NMuMG. She is also interested in cancer metabolism.

Tatsuya Ihara is a graduate student in Semba's lab, Waseda University. He received a Bachelor of Science degree from Waseda University in 2012. He is currently working on establishing cell lines which are suitable for assessment of tumorigenesis in mice.



Hiraku Suda is a graduate student in Semba's lab, Waseda University. He received a Bachelor of Science degree from Waseda University in 2013. He is currently screening for novel oncogenes using 3-dimensional culture of mammary epithelial cell line, MCF10A.



Hirofumi Mikami is a graduate student in Semba's lab, Waseda University. He received a Bachelor of Science degree from Waseda University in 2013. He is currently working on mammary stem cells. He is especially interested in technologies to manipulate mammary stem cells.

Kentaro Semba is a professor in the Department of Life Science and Medical Bioscience, Waseda University (2007-present). He received his PhD degree in Science from the University of Tokyo (1988). Thereafter, he was working as an assistant and associate professor in the Institute of Medical Science, the University of Tokyo (1988–2007). During his whole career, his interests are discovery of oncogenes, analysis of their function and contribution to diagnosis and treatment of cancer. Especially, his laboratory is focusing on the searching for oncogenes in the amplicons of breast cancer.