Review Article

Amplification of Cellular Oncogenes in Solid Tumors

Ozkan Bagci, Serkan Kurtgöz

Department of Medical Genetics, Suleyman Demirel University, School of Medicine, Isparta, Turkey

Abstract

The term gene amplification refers to an increase in copy number of a gene. Upregulation of gene expression through amplification is a general mechanism to increase gene dosage. Oncogene amplifications have been shown in solid human cancers and they are often associated with progression of cancer. Defining oncogene amplification is useful since it is used as a prognostic marker in clinical oncology nowadays, especially v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (*HER2*) targeted agents are used in breast cancer patients with high level of *HER2* overexpression as a therapeutic approach. However, patients without *HER2* overexpression do not appear to benefit from these agents. We concluded that determination of oncogene amplification in solid tumors is an important factor in treatment of human cancers with many unknowns. We have referred to PubMed and some databases to prepare this article.

Keywords: Amplification, oncogene, solid tumors

Address for correspondence: Dr. Ozkan Bagci, Department of Medical Genetics, Suleyman Demirel University, Medical Faculty, Isparta, Turkey.
E-mail: ozkanbagci@sdu.edu.tr

Introduction

Cancer originates from genomic alterations that occur in oncogenes, tumor suppressor genes, and micro-ribonucleic acid (miRNA) genes.[1] These changes are generally of somatic nature; while germline mutations result in susceptibility to familial cancers for individuals.[1] The earliest evidence indicating that cancers arise from somatic genetic alterations was obtained from the studies conducted on Burkitt's lymphoma, which reported that v-myc avian myelocytomatosis viral oncogene homolog (*MYC* proto-oncogene) displayed an oncogene activation as a result of the translocation between *MYC* proto-oncogene localized at q24 domain of chromosome 8 and one of three different chromosomes (14q, 22q, and 2p) bearing immunoglobulin loci.[1-3]

Functional Characteristics of Oncogenes

Oncogenes encode proteins that control cell proliferation, programmed cell death (apoptosis), or both. Proteins encoded by oncogenes are classified under six categories: Transcription factors, proteins remodeling chromatin structure, growth factors, growth factor receptors, signal transducers of signaling pathways, and apoptosis regulators [Table 1]. Proteins functioning as signal transducers of signaling pathways are further divided into two main classes of either nonreceptor protein kinases or guanosine triphosphate (GTP) binding proteins.[1,4,5]

Activation of Oncogenes

Oncogenes are activated by three mechanisms, namely, mutation, gene amplification, and translocation.[1,4,6] Activated oncogenes provide cells with oncogenic features such as increased cellular survival and proliferation.[7] Several studies have indicated that oncogene amplification frequently occurs in metastatic and low-differentiated tumors, implying that oncogene amplification takes place in a later stage of tumor

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Table 1: Proteins coded by oncogenes and certain types of cancer

Oncogene	Chromosome localization	Functional character of the protein	Type of cancer*
N-MYC	2p24	Transcription factor	Neuroblastoma, lung carcinoma
BCL-2	18q21.3	Anti-apoptotic	B-cell lymphoma
ALL1	11q23	Chromatin modification	Acute myeloid leukemia
HST	11q13.3	Fibroblast growth factor	Gastric carcinoma
EGFR	7p12	Epidermal growth factor receptor	Squamous cell carcinoma
SRC	20q12-q13	Nonreceptor protein tyrosine kinase	Colon carcinoma
H-RAS	11p15.5	GTPase activity	Colon, lung, and pancreas carcinoma

*Although oncogene activation results in many types of cancer, a limited number of examples are provided in this table. GTPase = Guanosine triphosphatase

development. On the other hand, translocations and mutations occur in the early stage or throughout the process of neoplastic transformation.[4,8]

Chromosomal arrangements

Translocations and associated gene fusions play a particularly significant role in the early stage of tumorigenesis; studies have thus far defined 358 gene fusions that involve 337 different genes. Whereas gene fusion and oncogene activation constitute an important diagnostic and prognostic parameter, particularly for malignant hematological diseases and childhood sarcomas, gene fusions have rather limited clinical and biological impact in solid tumors.[9] In prostate carcinoma, which is one of the few examples of such an impact, the fusion protein which emerges as a result of a translocation between the squalene monooxygenase gene *(ERG1)* associated with ETS transcription factor family and the transmembrane protease, serine 2 gene (*TMPRSS2*) triggers the carcinogenesis process by inhibiting apoptosis of the cells in the prostate gland and increasing cell proliferation.[10]

Mutations

When mutated, proteins coded by oncogenes assume a significant role in the cell's oncogenic transformation. The rat sarcoma gene *(RAS* proto-oncogene); which is activated by point mutation in many carcinomas such as lung, colon, pancreas, and thyroid carcinoma; was the first oncogene identified in human beings. Especially when mutated at codons 12, 13, and 61; *RAS* gene product result in a constant signal transduction due to serine/threonine kinases binding to tyrosine kinases. These constant signals, in turn, cause uncontrolled cell growth. The *RAS* gene family consists of the three interrelated members H-*RAS*, K-*RAS,* and N-*RAS*. While K-*RAS* mutations are often observed in lung, colon, pancreas, and thyroid carcinomas; N-*RAS* mutations are observed in myeloid leukemia and thyroid carcinoma and H-*RAS* mutations are frequently seen in thyroid carcinoma.[1,4,6,11,12] V-raf murine sarcoma viral oncogene homolog B gene *(BRAF* proto-oncogene), localized at chromosome 7q34, is another oncogene that is activated through point mutation; it codes a protein that belongs to the serine/threonine protein kinase family and functions on the mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK) pathway, which constitutes a significant mitogenic signal for cell proliferation. *BRAF* mutations often result in the valine residue located at position 600 to transform into glutamic acid. This transformation takes place at the kinase domain of BRAF protein and results in an uncontrolled activation of the MAP kinase/ERK signaling pathway. Mutations in *BRAF* proto-oncogenes are often related to colon cancer, malignant melanoma, hepatocellular carcinoma, and thyroid carcinoma.[13-15] Though there are more examples of oncogene activation through the mechanism of mutation in solid tumors, this study is limited to the two outlined above.

Gene amplification

It is now known that oncogene amplification contributes significantly to the development of many solid tumors in human beings. At the same time, oncogene amplification may reflect the genetic instability of solid tumor cells. Increased gene dosage through deoxyribonucleic acid (DNA) amplification constitutes a general genetic mechanism for the regulation of gene expression.[8,16,17] In organisms, gene amplification may occur through two different mechanisms, planned or unplanned. The former may be a part of the developmental stage of the embryo; the amplification of chorion genes in fruit fly ovaries or the amplification of actin genes during the development of chicken muscle tissues are two examples in this regard. The amplification displayed by mammals is the unplanned aberration which involves two unrelated mechanisms. Unplanned DNA amplification occurs in cellular response during tumor growth or from exposure to cytotoxic agents.[17,18] However, this amplification mechanism should not be confused with high gene expression, although it generally brings about an increase in the level of products coded through an amplified gene. Increased expression is often a result of amplification. Nevertheless, apart from amplification, there are alternative pathways to increase gene

expression. For instance, studies have demonstrated that although fibroblast growth factor 3 gene *(FGF3)* and *FGF4* oncogenes localized at 11q13 were observed to have been amplified in many solid tumors, they displayed a low level of expression.[18]

The phenomenon of gene amplification is sometimes observed as trisomy or tetrasomy for a certain chromosome; however, as is often the case with v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog gene (N-*MYC*) amplification in neuroblastoma, it is also observed as an amplified DNA, a homogeneously staining region (HSR), or as non-centromeric and non-telomeric extrachromosomal structures known as double minutes (DMs).[5,19,20] Various models have been suggested for the amplifications observed in solid tumors, but the amplification model characterized by the emergence of DM and HSR structures is the translocation-deletion-amplification model also called "the episome model" or "deletion-plus-episome". Additionally, the "breakage-fusion-bridge" (BFB) model is based on the *in situ* amplification of wide DNA regions through chromosomal recombination. Unlike these models, the chorion gene in Drosophila oocytes may amplify up to 60 times throughout the natural development process, which takes an onion-skin form and occurs through bi-directional openings of the replication fork. In this model, while amplicons grow in the number of copies towards the center, the sides display fewer copies.[21-23]

Amplified oncogenes in solid tumors often belong to three oncogenic gene families (*MYC*, *EGFR*, and *RAS*) or to 11q13 locus.[1,8] The amplification of an oncogene type usually does not pertain to a single tumor. However, the amplification of the N-*MYC* oncogene is frequently limited to neuronal-origin tumors, while v-myc avian myelocytomatosis viral oncogene lung carcinoma derived homolog gene (L-*MYC*) amplification is limited to lung carcinoma.[8] By contrast, many solid tumors display more than one oncogene amplification. This mechanism may occur on the same chromosome (syntenic) as well as on different chromosomes (non-syntenic).[18] For instance, in melanoma, fibroblast growth factor 4 (*HST*) and *INT2* oncogenes localized at 11q13 are amplified together.[24] Additionally, amplification of *MYC* (8q24), cyclin-dependent kinase 4 (*CDK4)*, and Mouse DM 2 homolog *(MDM2)* oncogenes localized (12q13-15) at different chromosomal areas has been observed in malignant salivary gland tumors.[25]

MYC oncogene family

The *MYC* oncogene family has three members: N-*MYC* (2p24.1), C-*MYC* (8q24), and L-*MYC* (1p34.3), each of which are localized at different chromosomes.

MYCN (2p24) amplification in neuroblastoma

MYCN amplification in neuroblastoma constitutes an important model for oncogene amplification in tumorigenesis. In other neuronal tumors such as retinoblastoma, glioblastoma, small cell lung carcinoma, and astrocytoma carcinoma, however, *MYCN* amplification has been identified with a lower frequency.[16,17,26] As demonstrated in Figure 1, amplified copies of oncogene *MYCN* can display an extrachromosomal structure as DMs and an intrachromosomal structure as HSRs.[16-18,26-28]

The fact that neuroblastoma cells contain *MYCN* as a single copy does not constitute sufficient evidence to suggest the absence of *MYCN* amplification. As shown by the fluorescent *in situ* hybridization (FISH) method, *MYCN* gene 2p24 can be duplicated as well. However, it is not yet clear whether the duplication is an introductory mechanism for amplification or an alternative pathway to activate the oncogenic potential of *MYCN*.[18]

The amplified *MYCN* gene also increases the risk for the amplification of some other similar genes. For instance, DEAD (Asp-Glu-Ala-Asp) box helicase 1 (*DDX1*) gene, localized at the 400 kb region on the fifth edge of the *MYCN* gene, is amplified in approximately 50% of *MYCN*-positive cases in both retinoblastoma and neuroblastoma. However, there is no case in which the *DDX1* gene is amplified on its own.[18,29,30] Further, recent studies have determined a correlation between the anaplastic lymphoma receptor tyrosine kinase genes (*ALK*) localized at 2p23 and *MYCN* amplification.[31,32] Sequence analyses found no mutation in the coding sequences of *MYCN* gene, indicating that an increased number of copies of the nonmutated gene (wild type) induces tumorigenesis.[18] *MYCN* amplification with a high number of copies is found in the aggressive variants

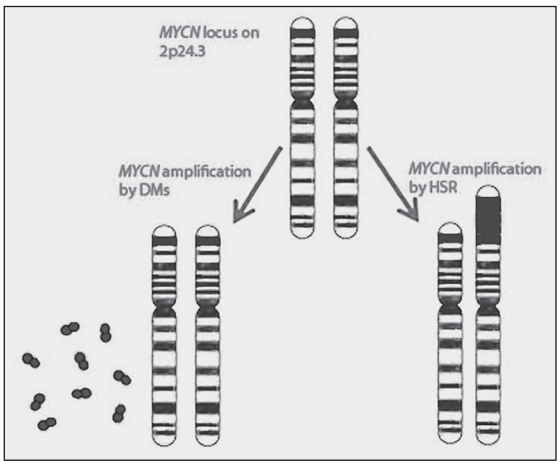

Figure 1: Two different amplification mechanisms for the *MYCN* oncogene[27]

of neuroblastoma. Therefore, it constitutes a powerful marker for the prognosis of the disease.[33,34]

EGFR oncogene family

The epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase family that includes ErbB2, ErbB3, and ErbB4. In many tumors, the regulation of these receptors is disrupted due to the overexpression of EGFR as a result of the amplification and mutation of the oncogene *EGFR*.[35]

ERBB2 (17q12-21) amplification in breast cancer

Amplification of *ERBB2*, which is also called *HER2*/neu, has been demonstrated in approximately 20-25% of breast cancer cases and is associated with bad prognosis. A similar amplification frequency has also been detected in ovarian cancer cases. Sequence analyses found no mutation in the coding sequences of the *ERBB2* gene, like the *MYCN* gene, which indicates that a high number of wild type gene copies plays a significant role in tumorigenesis.[16-18,36] Furthermore, studies have demonstrated *ERBB2* amplification in bladder carcinoma and squamous cell carcinoma of the uterine cervix.[37,38]

Oncogenes localized at chromosome 11q13

Although *FGF3 (INT2)*, *FGF4 (HST)*, and *CCND1 (CYD1)* oncogenes localized at chromosome 11q13 locus generally display a low (3-10 times) level of amplification, the 11q13 locus is amplified in many solid tumors. *INT2* and *HST* oncogenes are amplified together in many solid tumors such as melanoma, oral squamous cell carcinoma, breast carcinoma, and squamous cell carcinoma of the gallbladder.[18,21,39-41] Cyclin D1, which is another oncogene localized at the chromosome 11q13 locus, plays a significant role in G1/S transition in the cell cycle. Thus, since the amplification of cyclin D1 results in hyperproliferation, it contributes to tumorigenesis.[18] Further studies have reported amplified cyclin D1 in many solid tumors such as colon carcinoma, rectal adenocarcinoma, breast cancer, parathyroid adenoma, and prostate cancer.[42]

RAS oncogene family

H-*RAS*, K-*RAS*, and N-*RAS*; the three members of the *RAS* oncogene family; code a 21-kD protein that plays a significant role in the transmission of cellular growth signals. It has been demonstrated in many solid tumors that the cell enters the transformation phase due to the disrupted regulation of *RAS* protein as a result of the mutations occurring at codons 12, 13, and 60 of *RAS* genes.[43,44]

RAS genes become oncogenically activated not only by mutation but also through the mechanism of the amplified wild type gene, which implies that *RAS* oncogenes can contribute to both early and late phases of the carcinogenesis process.[44] Studies have reported amplified *RAS* genes in the early phases of lung carcinoma cases,[43] and studies conducted on 86 gastric tumors and 20 gastric cancer cell lines have demonstrated the amplification of K-*RAS*.[45] Another study on 31 colorectal carcinoma tumors reported H-*RAS* amplification in nine carcinomas and K-*RAS* amplification in seven carcinomas.[46]

In addition to the aforementioned oncogene groups, there is also the chromosome 12q13-15 region, which is frequently amplified in sarcomas. The oncogene *MDM2* localized at this region is amplified in the majority of sarcomas.[18] The protein MDM2 is related to p53, and the increase in MDM2 functions to reduce the tumor-suppressing activity of p53. This mechanism takes place through the protein MDM2 degrading the protein p53 by an E3 ubiquitin ligase activity.[47] Evidence supporting this mechanism is observed in neuroblastoma cases. p53 mutations are well-known in many different types of cancers. However, p53 mutations have not been detected in neuroblastoma cases; therefore, the protein p53 is considered to be inactivated by the increased expression of MDM2. Alternatively, MDM2 can activate itself oncogenically independent of p53, through a pathway that remains undefined.[17] A retrospective study of the literature analyzed 3,889 samples under 28 different tumors and demonstrated *MDM2* amplification in 19 types of tumor. The highest rates of amplification among tumors were displayed by soft tissue tumors (20%), osteosarcomas (16%), and esophageal carcinomas (13%).[48]

Conclusion

Amplified oncogenes are found in a broad spectrum of solid tumors and constitute a significant genomic power contributing to the development of solid tumors. Determining the amplification of oncogenes in tumor cells is important in both clinical and biological terms.

Today, most of the medications used for the treatment of cancer either cause DNA damage or prevent DNA replication, but the issue is that most of those medications produce a toxic effect on normal cells. An alternative method for the treatment of cancer could be drugs that directly target oncogenes that play an important role in tumorigenesis; although the fact that oncogenes are found as proto-oncogenes in normal cells constitutes a negative aspect of this treatment approach. Nevertheless, significant progress has been achieved with this treatment approach in some types

of cancer. In breast cancer cases, for instance, the protein ErbB2, a receptor tyrosine kinase, becomes overexpressed as a result of the amplification of *ERBB2* gene, as discussed above. Herceptin is a monoclonal antibody recognizing the ligand binding domain of the ErbB2 receptor tyrosine kinase. It has been reported that Herceptin binds to these overexpressed receptors and decelerates tumor development, prolonging the patients' lifetime. Therefore, determining oncogene amplification, which appears to be a challenge with multiple variables, constitutes an important factor in the treatment of cancer.

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Conflicts of interest

There are no conflicts of interest.

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