Amplification of Cellular Oncogenes in Solid Tumors

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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

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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
thyroid carcinoma and H-RAS mutations are observed in myeloid leukemia and RAS lung, colon, pancreas, and thyroid carcinomas; N-RAS is seen in thyroid carcinoma. [1,4,6,11,12] V-raf murine N-RAS of the three interrelated members H-RAS, K-RAS, and N-RAS plays 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]

### 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 | 18q12.1 | Anti-apoptotic | B-cell lymphoma |
| ALL1 | 11q23 | Chromatin modification | Acute myeloid leukemia |
| HST | 11q13.3 | Fibroblast growth factor | Gastric carcinoma |
| EGF | 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]
RAS 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.\[35\]

**EGFR oncogene family**

**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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