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Oncogenes as Markers for Early Detection of Cancer

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Abstract Oncogenes are formed in human tumors as a result of mutations or DNA rearrangements leading to the abnormal expression or function of proto-oncogenes. Approximately 20 different oncogenes are reproducibly activated in malignancies of several types, including breast, colon, lung, pancreatic, and thyroid carcinomas, leukemias, and lymphomas. The potential utility of these oncogenes as markers for early detection of cancer is dependent on the stage of tumor development at which they are activated, and on whether the mutated oncogenes are readily distinguished from the corresponding proto-oncogenes by assays that are sufficiently sensitive to detect precancerous lesions.

Key words: *abl*, chemoprevention, colorectal carcinoma, intermediate biomarker, leukemia, oncogenes, polymerase chain reaction, *ras*, tumor suppressor genes

It is now widely recognized that human tumors result from accumulated genetic damage leading to the activation of oncogenes and the loss or inactivation of tumor suppressor genes. Since mutations in these genes represent critical events in tumorigenesis, it is reasonable to expect that such mutations might also prove useful as markers of tumor development. This article will provide a brief review of oncogenes and discuss their potential application as early markers of neoplasia. In considering the applicability of oncogenes as markers of tumor development, at least three points need to be addressed: 1) How frequently are specific oncogenes activated in different types of tumors?, 2) At what stage of tumor development does oncogene activation occur?, and 3) How readily can oncogene activation be detected?

MECHANISMS OF CELLULAR ONCOGENE ACTIVATION

Oncogenes were first identified as specific genes of acutely transforming retroviruses that induced transformation of virus-infected cells. Subsequently, cellular oncogenes were identified by three approaches: (1) as homologs of retroviral oncogenes, (2) as genes that induce transformation upon introduction into appropriate recipient cells by transfection, and (3) as genes that are frequently altered in tumors by DNA rearrangement or amplification. Together, these approaches have identified more than 70 cellular genes that, as activated oncogenes, can induce at least some aspects of the tumorigenic phenotype [1].

Oncogenes are formed from normal cellular genes (proto-oncogenes) as a consequence of genetic alterations that result in either abnormal gene expression or the synthesis of altered proteins. In some cases, altered expression of a normal gene product is sufficient to convert a proto-oncogene to a biologically active oncogene. In other cases, the oncogene proteins differ in structure and function from those encoded by proto-oncogenes. The mechanisms by which oncogenes are activated in tumors is an important consideration in terms of their potential detection as early markers of tumor development.

One mechanism that can result in elevated expression of an oncogene is gene amplification, which results in an increased number of gene copies per cell. Elevated gene expression is then a direct result of an increased number of templates available for transcription. In this case, there are no distinct structural differences between the oncogene and the proto-oncogene. Consequently, detection of an amplified oncogene is possible only by quantitation of copy number in tumor cells.

Alternatively, abnormal gene expression can result from DNA rearrangements, such as chromosome translocations. The prototype

example of oncogene activation by this mechanism is translocation of the c-myc gene in Burkitt's lymphomas from its normal locus on chromosome 8 to one of the immunoglobulin gene loci on chromosomes 2, 14, and 22 [2]. This results in a loss of normal gene regulation, leading to constitutive expression of the normal c-myc protein. The translocations resulting in activation of c-myc, and other oncogenes that are deregulated in a similar manner, can occur over a broad region of DNA. Thus, like gene amplification, these translocations do not result in the formation of a distinct molecular marker of oncogene activation.

Other translocations, however, result in the formation of oncogenes that have suffered reproducible structural alterations, leading to formation of an altered gene product. These oncogenes encode recombinant fusion proteins, formed by recombination between coding sequences of two distinct genes. The activation of the abl oncogene by the Philadelphia translocation in chronic myelogenous leukemia is an example of such a DNA rearrangement [3]. In this translocation, the abl proto-oncogene from chromosome 9 recombines with another gene, bcr, on chromosome 22. As a result of this rearrangement, the amino-terminal sequences of abl are deleted and replaced with bcr coding sequences. The recombinant bcr/abl oncogene protein functions in an uncontrolled manner, leading to the development of neoplasia. Since the recombination event occurs in a defined region of both the abl and bcr genes, the recombinant transcript represents a unique fusion between abl and bcr sequences. Consequently, the oncogene bcr/abl mRNA can be sensitively detected using polymerase chain reaction (PCR) primers that span the recombination site [4]. Similar DNA rearrangements lead to the activation of several other oncogenes in human tumors, including the retinoic acid receptor in acute promyelocytic leukemias [5,6].

Other oncogenes are activated by point mutations rather than DNA rearrangements. The ras oncogenes, for example, are activated by point mutations leading to single amino acid substitutions at critical positions in the ras gene products [7]. Such single amino acid substitutions result in deregulation of ras protein function, converting a normally regulated proto-oncogene protein into an oncogene protein that is constitutively active. Similar point mutations are responsible for activation of the gsp and gip oncogenes in some hormone-responsive human tumors [8]. Such point mutations can also be sensitively detected by PCR analysis, using mutation-specific PCR primers or oligonucleotide probes [9].

ONCOGENE FUNCTIONS

Most proto-oncogenes are normally expressed in a wide variety of cell types, where they function to regulate normal cell proliferation in response to mitogenic stimuli. The proteins encoded by almost all of the characterized oncogenes can be divided into five functional groups, which act as regulatory elements in signal transduction pathways leading to cell proliferation (Table 1) [10]. Whereas the proto-oncogene products function in normal cell proliferation, the unregulated expression or activity of the oncogene proteins leads to a loss of normal growth control and tumor development.

First, a number of oncogenes encode extracellular growth factors. The prototype of this group of oncogenes is sis, the oncogene of simian sarcoma virus, which encodes the B chain of platelet-derived growth factor. Other members of this group of oncogenes include several members of the fibroblast growth factor family and hematopoietic growth factors. Transformation by these oncogenes is a consequence of abnormal growth factor expression by a responsive cell, leading to autocrine stimulation of cell growth.

The second major group of oncogenes encode protein-tyrosine kinases. There are two classes of these proteins. The receptor protein kinases are membrane-spanning molecules that function as growth factor receptors. The erbB oncogene, which is derived from the EGF receptor, is the prototype of this group. A closely related gene, erbB-2, is frequently amplified in human breast and ovarian carcinomas [11]. Other receptor protein-tyrosine kinases include ret and trk, which are frequently activated in human thyroid carcinomas [12,13]. The nonreceptor protein-tyrosine kinases, including abl, are intracellular molecules. Many of these proteins are associated with the inner face of the plasma membrane where they may function in noncovalent association with cell surface receptors.

The third group of oncogene proteins, guanine nucleotide binding proteins,

TABLE I. Oncogene Functional Groups

Functional Activity	Representative Oncogenes
Growth Factors	sis, FGF, int-1, int-2, hst, fgf-5, IL-2, IL-3, CSF-1, GM-CSF
Protein-Tyrosine Kinases	
Receptor	erbB, erbB-2, fms, ros, trk, met, kit, ret, sea
Nonreceptor	src, yes, fgr, lck, fyn, lyn, hck, abl, fes
GTP Binding	rasH, rasK, rasN, gip, gsp
Protein-Serine/Threonine Kinases	mos, pim-1, c-raf, A-raf, B-raf
Transcription Factors	erbA, c-jun, jun-B, jun-D, c-fos, fra-1, fos-B, c-myc, L-myc N-myc, myb, ets, E2A, RAR, rel

includes the ras gene products, which are the oncogenes most frequently activated in human tumors [14]. The ras proteins are localized to the inner face of the plasma membrane and are thought to function in signal transduction from growth factor receptors to second messengers, which still remain unidentified in mammalian cells. The activity of the ras proteins is controlled by GTP/GDP binding and hydrolysis, analogous to the G proteins that serve to regulate adenylate cyclase and other enzymes affecting the metabolism of intracellular second messengers [15,16]. The genes encoding Gs and Gi, gsp and gip, also act as oncogenes in some hormone-responsive cells, such as ovarian and pituitary tumors.

Other oncogenes encode protein-serine/threonine kinases that are cytosolic enzymes. These oncogenes include members of the raf family, which are activated in response to growth factor stimulation of a variety of cell types [17].

Finally, a large number of oncogenes encode nuclear proteins, many of which have been shown to function as transcriptional regulatory factors [18]. These include the fos and jun oncogene products, which comprise the AP-1 transcription factor, as well as members of the myc gene family, which are frequently activated by DNA rearrangement or gene amplification in a variety of human neoplasms. The erbA oncogene, which encodes thyroid hormone receptor, and the RAR oncogene, which encodes retinoic acid receptor, are also members of this group.

Most oncogene products can thus be viewed as regulatory elements in intracellular signal transduction pathways leading to cell proliferation. Extracellular growth factors act to stimulate the enzymatic activity of receptor protein-tyrosine kinases, which then transmit a mitogenic signal via activation of ras gene products, the raf protein-serine/threonine kinase, and phospholipase C, resulting in formation of diacylglycerol and activation of protein kinase C. The activity of these cytosolic protein-serine/threonine kinases ultimately affects the activity and expression of transcriptional regulatory proteins in the nucleus, leading to changes in gene expression and cell division.

ONCOGENES IN HUMAN TUMORS

Although more than 70 oncogenes have been identified, not all of these are frequently encountered in human neoplasms. Reproducible activation of about 20 oncogenes has so far been described in human tumors, and these genes, which represent potential markers of human neoplasia, are indicated in Table 2.

Some of these oncogenes are activated highly reproducibly in specific types of tumors, and their activation appears to play a role in the genesis of nearly all individual neoplasms of these types. Such oncogenes include abl in chronic myelogenous leukemia, bcl-2 in follicular B-cell lymphomas, c-myc in Burkitt's lymphomas, and the retinoic acid receptor (RAR) in acute promyelocytic leukemia. Other oncogenes are activated in only a fraction of individual neoplasms of the types of tumors in which they are involved, including the ras genes in colon and lung carcinomas. In some

TABLE II. Oncogenes Activated in Human Tumors

Oncogene	Neoplasm	Activation Mechanism
abl	chronic myelogenous leukemia	translocation
	acute lymphocytic leukemia	
bcl-2	follicular B-cell lymphoma	translocation
bcl-3	chronic B-cell leukemia	translocation
can	acute nonlymphocytic leukemia	translocation
E2A	acute lymphocytic leukemia	translocation
erbB-2	breast and ovarian carcinoma	amplification
gip	adrenal cortical and ovarian carcinoma	point mutation
gli	glioblastoma	amplification
gsp	pituitary carcinomas	point mutation
hox-11	acute lymphocytic leukemia	translocation
lyl	acute lymphocytic leukemia	translocation
c-myc	Burkitt's lymphoma	translocation
	breast and lung carcinoma	amplification
N-myc	neuroblastoma, lung carcinoma	amplification
L-myc	lung carcinoma	amplification
RAR	acute promyelocytic leukemia	translocation
rasH	thyroid carcinoma	point mutation
rasK	colon, lung, pancreatic, and thyroid carcinomas	point mutation
rasN	acute myeloid and lymphoid leukemia thyroid carcinomas	point mutation
ret	thyroid carcinoma	rearrangement
rhom	acute lymphocytic leukemia	translocation
scl	acute stem cell leukemia	translocation
tan	acute lymphocytic leukemia	translocation
trk	thyroid carcinoma	rearrangement

cases, activation of these oncogenes is correlated with differences in tumor behavior. For example, amplification of N-myc in neuroblastomas is found in more aggressive tumors and is correlated with progression to increasing malignancy [19]. Amplification of erbB-2 is similarly correlated with the malignancy of breast and ovarian carcinomas [11].

Different oncogenes are often involved in different stages of tumor development. Carcinogenesis is clearly a multistep process, which frequently occurs as a consequence of accumulated damage to both oncogenes and tumor suppressor genes. Some oncogenes are involved in early stages of tumorigenesis, whereas others appear to be involved in later stages of tumor progression. In several types of neoplasms, ras oncogenes appear to play a role in early stages of tumorigenesis. For example, ras oncogenes are activated by mutations characteristic of those induced by the initiating carcinogen in a variety of experimental animal tumors, suggesting that ras genes are targets for carcinogen-induced mutations at the initiation stage of tumor development [7]. Likewise, ras oncogenes are activated at early stages of the development of several human neoplasms. In colorectal carcinomas, for example, activation of rasK and inactivation of the APC and MCC tumor suppressor genes appear to be early events leading to the development of premalignant adenomas, whereas inactivation of the DCC and p53 tumor suppressor genes usually occurs at later stages of progression to malignancy [20]. Activation of ras oncogenes similarly appears to occur as an early event, preceding malignancy, in thyroid carcinomas and some leukemias [7,14]. In addition, ras oncogenes are characterized by different mutations in different types of cancers (e.g., colon and lung carcinomas), suggesting that ras genes may be targets for carcinogen-induced mutations in these human tumors as well as in experimental animal neoplasms [14]. Detection of ras oncogene mutations may therefore provide a marker for early stages of development of a significant fraction of human cancers.

In contrast, other oncogenes appear to be involved in later stages of tumor progression. These oncogenes include N-myc in neuroblastomas; c-myc, N-myc, and L-myc in lung carcinomas; and erbB-2 in breast and ovarian carcinomas. Since these oncogenes are activated relatively late in tumor progression, they do not constitute markers suitable for early detection.

EARLY DETECTION OF HUMAN TUMORS

In solid tumors, the ras genes provide the most likely early detection markers, being frequently activated in colon, lung, pancreatic, and thyroid carcinomas. In addition to their activation at early stages of tumor development, the mutations responsible for ras oncogene formation can be readily detected in small amounts of material using PCR amplification [9]. For example, it has been possible to detect ras mutations in mammary glands two weeks after exposure to the chemical carcinogen nitrosomethylurea, well before the onset of neoplasia [21]. The sensitivity of current methods for detection of mutations in ras has been estimated to be sufficient to detect one mutant gene in the presence of 10^5 normal alleles [9]. Thus, analysis of mutant ras genes provides a sensitive assay for early events in carcinogenesis. The erbB-2 oncogene, in contrast, is amplified at late stages of tumor progression, as discussed above. Moreover, since its activity as an oncogene results from gene amplification rather than from distinct mutations, sensitivity of detection would pose a problem.

The ras oncogenes might also be used for early detection of some acute leukemias. In these hematopoietic neoplasms, however, a number of other oncogenes have also been identified [22]. In several cases, these genes are activated by chromosome translocations that result in formation of recombinant fusion proteins. Examples include the activation of abl in chronic myelogenous leukemia and RAR in acute promyelocytic leukemia [3,5,6]. These oncogenes can be detected with high sensitivity by PCR analysis of cDNAs using primers that span the recombination sites joining sequences that were unlinked in normal cells--for example, the recombination site between bcr and abl sequences in the bcr/abl recombinant transcript. Detection of this rearrangement is already being used to monitor recurrence of leukemia following treatment of chronic myelogenous leukemia patients [4], and could provide an assay suitable for early detection as well. Thus, in the leukemias and lymphomas, a number of oncogenes activated as recombinant fusion proteins are candidate markers for early disease detection.

REFERENCES

1. Cooper GM: Oncogenes. Jones and Bartlett Publishers, Boston. 1990.
2. Leder P, Battey J, Lenoir G, Moulding C, Murphy W, Potter H, Stewart T, Taub R: Translocations among antibody genes in human cancer. Science 222:765-771, 1983.
3. Shtivelman E, Lifshitz B, Gale RP, Canaani E: Fused transcript of abl and bcr genes in chronic myelogenous leukemia. Nature 315:550-554, 1985.
4. Sawyers CL, Timson L, Kawasaki ES, Clark SS, Witte ON, Champlin R: Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. Proc Natl Acad Sci USA 87:563-567, 1990.
5. Borrow J, Goddard AD, Sheer D, Solomon E: Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. Science 249:1577-1580, 1990.
6. de The' H, Chomienne C, Lanotte M, Degos L, Dejean A: The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus. Nature 347:558-561, 1990.
7. Barbacid M: ras genes. Ann Rev Biochem 56:779-827, 1987.
8. Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh QY, Clark OH, Kawasaki E, Bourne HR, McCormick F: Two G protein oncogenes in human endocrine tumors. Science 249:655-659, 1990.
9. Kumar R, Barbacid M: Oncogene detection at the single cell level. Oncogene 3:647-651, 1988.
10. Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S: Oncogenes and signal transduction. Cell 64:281-302, 1991.
11. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707-712, 1989.
12. Grieco M, Santoro M, Berlingieri MT,

Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Porta G, Fusco A, Vecchio G: PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 60:557-563, 1990.

13. Bongarzone I, Pierotti MA, Monzine N, Mondellini P, Manenti G, Donghi R, Pilotti S, Grieco M, Santoro M, Fusco A, Vecchio G, Della Porta G: High frequency of activation of tyrosine kinase oncogenes in human papillary thyroid carcinoma. Oncogene 4:1457-1462, 1989.
14. Bos JL: ras oncogenes in human cancer: a review. Cancer Res 49:4682-4689, 1989.
15. Bourne HR, Sanders DA, McCormick F: The GTPase superfamily: conserved structure and molecular mechanism. Nature 349:117-127, 1991.
16. Bourne HR, Sanders DA, McCormick F: The GTPase superfamily: conserved switch for diverse cell functions. Nature 348:125-132, 1990.
17. Morrison DK, Kaplan DR, Escobedo JA, Rapp UR, Roberts TM, Williams LT: Direct activation of the serine/threonine kinase activity of raf-1 through tyrosine phosphorylation by the PDGF ß receptor. Cell 58:649-657, 1989.
18. Lewin B: Oncogenic conversion by regulatory changes in transcription factors. Cell 64:303-312, 1991.
19. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, Hammond D: Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med 313:1111-1116, 1985.
20. Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61:759-767, 1990.
21. Kumar R, Sukumar S, Barbacid M: Activation of ras oncogene preceding the onset of neoplasia. Science 248:1101-1104, 1990.
22. Rabbitts TH: Translocations, master genes, and differences between the origins of acute and chronic leukemias. Cell 67:641-644, 1991.