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Advances in Molecular Genetics

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Ample evidence has been discovered recently to support the fundamental concept that cancer is a genetic disease. Cancer results from the accumulation of genetic damage within the nucleus of a cell that ultimately causes transformation to a neoplastic phenotype. In patients with a familial cancer syndrome, a predisposition to the development of cancer is conferred by the inheritance from one parent of a mutated gene in the germline. Alternatively, in patients without a familial predisposition, somatic mutations within an individual cell give rise to transformation. A current model for neoplastic transformation of a cell is the hypothesis that cancer results from a stepwise accumulation of multiple genetic defects, resulting in the acquisition of increasingly more aggressive and uncontrolled growth properties.[1]

Genetic alterations in one of three broad categories of genes have now been connected to cancer development. These include proto-oncogenes, tumor suppressor genes, and most recently, genes that normally function in DNA damage recognition and repair (Table). Mutation in one allele of a proto-oncogene, the normal cellular counterpart of this gene, results in an oncogene with abnormal function or inappropriate expression. A mutation in one of these genes results in a gain-of-function, which is deleterious for cellular growth regulation, and the mutated allele thus confers oncogenic potential in a dominant fashion. By contrast, tumor suppressor genes encode products that normally exert negative control or a brake on cellular growth and division, or are integrally involved in programmed cell death (apoptosis). Oncogenic activity requires mutation, inactivation, or loss of both alleles (on the paternally and maternally derived chromosomes), and results in a complete loss-of-function and presumably unregulated cellular growth and proliferation. Most recently, loss-of-function mutations in a third category of genes that normally function in DNA damage recognition and repair (DNA mismatch repair genes) have been associated with colorectal and other cancers in the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, as well as a significant number of sporadic colorectal cancers.

Familiarity with the mechanisms of tumorigenesis and known genetic defects in human neoplasms assumes increasing importance for the surgical oncologist. Molecular genetic analysis currently provides for accurate predictive testing in patients at high risk for cancer development in a variety of familial cancer syndromes, allows prognostic stratification by identifying specific genetic defects that are associated with increased potential for tumor aggressiveness or metastases, and holds promise for future directed therapeutic interventions.

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GENETICS OF COLORECTAL NEOPLASIA

Colorectal cancer provides an ideal model to study tumorigenesis because of the existence of distinct hereditary syndromes associated with a high risk for the development of colon cancer and the stepwise transition from adenoma to carcinoma proposed for sporadic cancers.

Familial Adenomatous Polyposis (FAP)

Familial adenomatous polyposis is a rare autosomal dominant cancer syndrome in which affected individuals develop 100 or more adenomatous polyps of the colon by the third or fourth decade of life, and subsequently carry a very high risk of developing a colorectal malignancy. Tumors in patients with FAP probably account for less than 0.5% of all colorectal cancers. However, this clinical phenotype is important because the predisposition to neoplasia of the colonic mucosa is transmitted by the inheritance of a germline mutation in the adenomatous polyposis coli (APC) tumor suppressor gene located on the long arm of chromosome 5. In the majority of patients with FAP, one mutant allele of APC has been identified in the constitutional (germline) DNA. In most cases, the defect is either a nonsense mutation or an insertion or deletion resulting in a truncated and thus inactive APC product. A second somatic event involving mutation or loss (allelic deletion) of the remaining APC allele within a somatic cell results in tumor formation.

The APC gene encodes a 2,843–amino acid product that is present in the cytoplasm bound to the adherens junction proteins α- and β-catenin. It has been postulated that loss-of-function mutations in both APC genes may disrupt normal epithelial cell-to-cell interaction.

Importantly, somatic mutations of APC can be detected in tumor DNA from more than two-thirds of sporadic colorectal neoplasms. Therefore, in addition to causing a rare familial syndrome when inherited as a germline mutation, the APC gene is an important target for oncogenic mutations in the common sporadic variety of colorectal cancer.

Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

Tumors arising in patients with the hereditary nonpolyposis colorectal cancer syndromes may account for 5–10% of cancers of the colon and rectum. Affected patients do not develop polyposis, but carry a high risk of developing colorectal cancers as well as other neoplasms (eg, endometrial carcinoma). Colon cancers in these individuals are

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TABLE 1
Genes, Gene Function and Tumor Development

Mechanism	Function	Gene	Tumor or Syndrome
Oncogene	Growth	*RET*	MEN 2A & B
		RAS	Colon cancer
			Pancreatic cancer
Tumor suppressor gene	Brake	*TP53*	Li-Fraumeni syndrome
			Colon cancer
		APC	FAP
		CDKN2	Pancreatic cancer
			Melanoma
		DCC	Metastatic colon cancer
		DCP4	Pancreatic cancer
Mismatch repair gene	DNA Repair	*MSH2*	Muir-Torre Syndrome
		MLH1	HNPCC
		PMS1	
		PMS2	

most commonly in the ascending colon. It has recently been demonstrated that patients with HNPCC have germline mutations in one of four DNA mismatch repair genes (*MSH2, MLH1, PMS1, PMS2*) that normally function in DNA damage recognition and repair. In approximately 60% of families with the clinical characteristics of HNPCC, a germline mutation is present in the *MSH2* gene on chromosome 2. A somatic mutation that inactivates the remaining normal allele occurs in tumor DNA, resulting in loss of function of the gene and decreased surveillance and repair of DNA mismatches that arise in a dividing cell. Tumor DNA from affected patients displays a characteristic replication error of repeats (RER) phenotype manifested as instability of simple sequence repeats. Some of these errors may result in further mutations that activate oncogenes or inactivate tumor suppressor genes. Importantly, approximately 20% of sporadic colorectal cancers display a RER^+ phenotype, suggesting a possible role for mutations in mismatch repair genes in the common sporadic variety of colon cancer.

Genetic Alterations in Sporadic Colorectal Neoplasms

Colorectal neoplasia is perhaps the most well-studied tumor system with respect to the model of multistep tumorigenesis.[1] Sporadic colorectal neoplasms spanning the spectrum from adenoma to carcinoma have been shown to harbor mutations or deletions affecting a variety of oncogenes and tumor suppressor genes that appear to play critical roles in oncogenesis. Somatic mutations in the *APC* gene are prevalent in colorectal carcinomas as well as early adenomas, suggesting that *APC* mutation may be an early or initiating event in tumorigenesis. Mutations in codons 12 and 13 of K-*RAS* are present in approximately 50% of adenomas greater than 1 cm in size and 50% of carcinomas, and are detected with increased frequency in adenomas with dysplasia. The association of K-*RAS* mutations with size and dysplasia suggests a role for *RAS* mutations in the progression from adenomatous polyp to dysplasia or carcinoma.

Chromosomal deletions in tumor DNA that are presumed to result in loss of the wild type allele of a tumor suppressor gene include allelic loss of the *TP53* gene on 17p and the *DCC* (deleted in colorectal carcinoma) gene on 18q. Chromosome 17p losses are infrequent in adenomas, but are present in more than 75% of carcinomas. In nearly all tumors with loss on one chromosome at 17p, inactivating mutations of the remaining *TP53* allele can be detected. The p53 product normally has a role in cell cycle control, and loss of function by inactivation of both copies of the gene appears to be a late event associated with transition to carcinoma. Chromosome deletions involving 18q are present in about 50% of late adenomas and 70% of carcinomas. Interestingly, virtually all colorectal tumors that have metastasized to the liver have 18q losses, suggesting a role for the *DCC* gene in tumor progression and the development of metastatic potential. The *DCC* gene encodes a protein product with sequence similarity to the neural cell adhesion molecule (NCAM) family of proteins, and loss of function of this tumor suppressor gene may adversely affect cell-to-cell interaction.

Potential Clinical Applications of Molecular Genetic Information in Patients with Colorectal Cancer

Knowledge of some of the molecular genetic alterations present in colorectal neoplasms has provided current and potential future clinical applications to the care of patients with colon cancer or at high risk for the development of colon cancer in the setting of a familial cancer syndrome. Molecular analysis may be applied to cancer screening, early detection, and prognostic stratification based on the mutational profile of a specific tumor. In the future, information about specific molecular defects may potentially be applied to the development of targeted therapeutic interventions or chemopreventative agents. Currently, the ability to detect germline mutations in patients at high risk for colon cancer in the setting of the FAP or HNPCC syndromes allows for predictive genetic testing prior to the development of neoplasia. In patients who have inherited a germline mutation, heightened surveillance and appropriate surgical treatment may be provided, whereas patients who are genetically negative may be spared the anxiety and expense of repeated colonoscopy and clinical testing. Although significant practical and ethical issues exist for the widespread institution of molecular analysis for screening and early cancer detection of sporadic neoplasms, it has been demonstrated that *RAS* mutations are detectable by polymerase chain reaction (PCR) amplification of cells shed from large adenomas or carcinomas into the stool.[2]

Characterization of tumor DNA at the molecular level may be increasingly applied to prediction of outcome and therefore treatment planning. For instance, it has been demonstrated that chromosome 18q loss (*DCC*) is associated with metastatic potential of colorectal neoplasms, and with a poorer 5-year survival compared stage for stage with tumors that do not have 18q loss.[3] Finally, increased understanding of the molecular pathogenesis of colorectal neoplasia holds promise for the development of novel treatment strategies. Already, small molecule inhibitors of *RAS* function have been shown to have some efficacy against

cancer cells harboring a *K-RAS* mutation.[4] It is likely that molecular genetic analysis will play an increasingly important role in diagnosis and treatment of solid human tumors in the future.

GENETICS OF THE MULTIPLE ENDOCRINE NEOPLASIA (MEN) TYPE 2 SYNDROMES

MEN 2A is inherited as an autosomal dominant disorder and is composed of medullary thyroid carcinoma (MTC), pheochromocytoma(s), and parathyroid hyperplasia. Virtually 100% of individuals who have this disorder develop MTC that subsequently can be lethal. The MEN 2A syndrome is associated with germline mutations in the *RET* proto-oncogene, a receptor tyrosine kinase that maps to the centromere region of chromosome 10. These missense mutations involve one of five codons in either exon 10 or 11 (codons 609, 611, 618, 620, 634), each of which specifies a highly conserved cysteine residue in the extracellular portion of the molecule immediately adjacent to the transmembrane domain (Figure). The mutations result in replacement of a cysteine residue with one of several other amino acids. Mulligan and coworkers[5] have studied the relationship between genotype and phenotype in patients with MEN 2A and have recognized that a significant correlation exists between any mutation at codon 634 and the development of pheochromocytomas. In addition, a strong correlation is present between the development of parathyroid disease and the specific mutation TGC > CGC (cysteine to arginine) at codon 634.

Familial medullary thyroid carcinoma (FMTC) is only associated with the autosomal dominant inheritance of medullary thyroid carcinoma and no other endocrinopathies. The MTC in this disease is most indolent and patients seldom die from it. It is associated with missence mutations in both the extracellular and intracellular domain of *RET* (Figure).

MEN 2B is also inherited as an autosomal dominant disorder and is caused by a mutation of *RET*, but this disorder is composed of a slightly different complex of diseases than MEN 2A and FMTC. Individuals with MEN 2B have a characteristic appearance that includes mucosal neuromas, prognathism, Marfanoid habitus, bony abnormalities, intestinal ganglioneuromatosis, and corneal nerve hypertrophy. These patients all develop an aggressive form of MTC; most develop pheochromocytomas, while none develop parathyroid disease. Virtually all patients with the rare MEN 2B syndrome have been shown to have an identical mutation at codon 918 in the tyrosine kinase catalytic domain of *RET* that results in substitution of a threonine for a methionine (Figure).

The *RET*-MEN 2A mutant allele results in receptor dimerization and autophosphorylation of the protein with tyrosine kinase activation,[6] which presumably can confer tissue-specific neoplasia of the thyroid C-cells, the chromaffin cells of the adrenal medulla, and more variably of the parathyroid glands. The MEN 2B mutation in the catalytic domain of *RET* has been postulated to alter the catalytic properties or substrate specificity of the tyrosine kinase portion of the receptor, which has been shown to result in receptor autophosphorylation approximately one-third to one-fifth that of *RET*-MEN 2A mutant allele.[6] Thus different mutations of the same gene result in different, although similar, diseases.

Early Thyroidectomy in Patients with MEN 2A Based on Genetic Testing

The detection of elevated plasma levels of calcitonin after stimulation with intravenous calcium and pentagastrin provides a sensitive method for the diagnosis of MTC in individuals from kindreds with MEN 2A who are at risk for the development of MTC. However, the biochemical screening of kindred members at risk for familial MTC requires annual provocative testing, which is associated with considerable unpleasant side effects. Direct DNA testing of peripheral leukocytes for mutations in *RET* of individuals at risk for one of the MEN 2 syndromes may now be routinely performed at any age, providing an opportunity for definitive and potentially curable surgical intervention based on a molecular genetic diagnostic test. Patients who have inherited a mutation in *RET* may be treated by early thyroidectomy at an age when the MTC is confined to the thyroid and likely curable. Our group has reported the results of 13 patients undergoing early thyroidectomy based on the results of direct DNA testing for MEN 2A.[7] All patients inheriting a RET mutation had evidence of C-cell neoplasia (C-cell hyperplasia, MTC, or both) in the resected thyroid gland. In this group of 13 patients, there were no metastases to regional lymph nodes, and postoperative stimulated plasma CT levels were normal in each patient. Thus, the surgery was not only prophylactic, but therapeutic, because it was able to remove carcinoma confined to the thyroid gland for cure. Further, individuals at risk who do not inherit a mutation of *RET* do not need additional testing as they will not develop the disease.

PANCREATIC CANCER

Pancreatic cancer is a highly lethal neoplasm. Few, if any, individuals are currently cured. Each year in the United States approximately 28,000 patients die from pancreatic cancer. Three different gene mutations have been associated with pancreatic cancer: *K-RAS*, *DPC4*, and *CDKN2*. *K-RAS* is an oncogene that is mutated in approximately 80% of pancreatic cancers and is associated with tumor progression in adenocarcinomas. *DPC4*, deleted in pancreas cancer locus 4, is a tumor suppressor gene that is located on the long arm of chromosome 18. This gene is missing in approximately 30% of pancreatic cancer specimens. The exact mechanism of *DPC4*'s effect on cell growth is unknown, but sequence homology to other genes suggests that it works through the TGF-B signaling pathway.[8] *CDKN2*, also known as *MTS1*, is a tumor suppressor gene that is known to be mutated or lost in 80% of pancreatic cancer cell lines and 40% of primary pancreatic cancers. Its exact function is unknown, but it is associated with one of the tyrosine kinase membrane receptors. *CDKN2* mutations may be able to identify individuals with familial melanoma who are at risk for the development of pancreatic cancer. Inherited *CDKN2* mutations have been reported in a family with both melanoma and pancreatic cancer.[9] *CDKN2* mutations in pancreatic cancer specimens are also associated with a poorer prognosis and decreased survival.[10] Thus, beside tumor size and the detection of positive lymph node metastases, which are known classic poor prognostic vari-

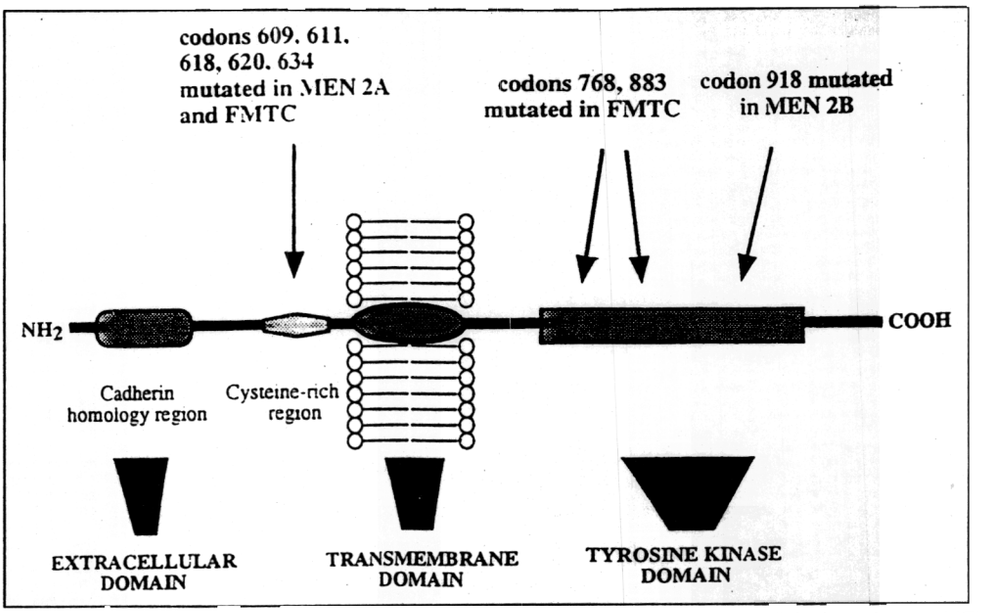

Figure. Different germline mutations in the *RET* proto-oncogene are associated with the distinct clinical syndromes MEN 2A, MEN 2B, and FMTC. The *RET* protein product is a receptor tyrosine kinase, schematically depicted by the solid line in the figure. The major domains of the *RET* product are represented by the shaded figures. Missense mutations that result in the substitution of a different amino acid for one of five highly conserved cysteines in the extracellular domain immediately adjacent to the transmembrane portion of the protein are associated with MEN 2A and some FMTC families. Some FMTC families have been shown to have mutations in codons 768 and 883 in the intracellular portion of the protein. Virtually all patients with MEN 2B have an identical mutation in codon 918 of the putative tyrosine kinase catalytic domain that results in the substitution of a threonine for a methionine.

ables, molecular changes within the tumor can be another detrimental variable.[10]

Diagnosis and Prognosis

These studies provide important new findings for surgeons involved in the management of patients with pancreatic cancer. Since a family history of melanoma can be associated with pancreatic cancer,[9] patients with pancreatitis have a greater risk of pancreatic cancer, and nearly all pancreatic tumors will have a mutation in either K-*RAS*, *DPC4*, or *CDKN2*, it may be possible to make the diagnosis earlier in individuals at risk by screening stool or pancreatic drainage specimens for the presence of specific gene mutations. In many centers studies are currently underway to assess the detection of genetic changes for early diagnosis. Further, in patients with a diagnosis of pancreatic cancer, important prognostic information can be ascertained by the detection of a mutation in *CDKN2*.

BREAST CANCER

Approximately 5–10% (9–18,000 cases per year in the United States) of breast cancer cases are caused by the autosomal dominant inheritance of a mutated gene. Forty-five percent of inherited breast cancers are due to *BRCA1*. Thirty-five percent of inherited breast cancers are due to *BRCA2*. Breast cancer is a feature of the Li-Fraumeni syndrome (LFS), hereditary early onset breast cancer syndrome, breast-ovarian cancer syndrome, Cowden's disease, Muir-Torre syndrome, and Peutz-Jeghers syndrome.

Li-Fraumeni Syndrome

Breast cancer is the most frequent manifestation of the LFS. Individuals may also develop sarcomas, brain tumors, laryngeal cancer, leukemia, and adrenal cancer. LFS can be caused by a germline mutation of the *TP53* gene.[11] However, not all individuals with LFS have mutations of *TP53*, and other mechanisms have been implicated such as post-translation protein modifications. *TP53* encodes a nuclear phosphoprotein whose normal function is to respond to DNA damage by blocking the cell cycle or inducing apoptosis. The absence of an inhibitory effect of p53 protein following DNA injury contributes to cellular transformation by allowing accumulation of unrepaired DNA mutations.

Hereditary Early-onset Breast Cancer and Breast-Ovarian Cancer Syndrome

Hereditary early-onset breast cancer syndrome is associated with a genetic abnormality on the long arm of chromosome 17. The disease gene locus was further refined to the 17q21 chromosomal region and the gene was subsequently cloned and sequenced as *BRCA1*.[12] *BRCA1* is a large protein with 1,863 amino acids and includes a zinc finger motif that appears to be involved with transcriptional regulation. However, the exact function of this gene is not yet known. There have been a large number of mutations in different alleles of *BRCA1* identified. Different alleles are associated with a different risk for the development of breast and ovarian cancer. Subsequently, it has been shown that only 45% of hereditary early onset breast cancers are associated with *BRCA1* mutations. In 1994 linkage studies demonstrated that another gene is involved that maps to 13q12–13.[13] Seventy percent of hereditary breast cancers not linked to *BRCA1* are linked to this other site, so called *BRCA2*. Cloning of the *BRCA2* gene has allowed direct

genetic testing for individuals at risk.[14] Women with either a *BRCA1* or *BRCA2* mutation have a significantly increased risk for the development of breast cancer: approximately 50% by age 45, 85% lifetime, risk of second breast cancer 65%, and 30% of women who develop breast cancer under the age of 35 will have inherited susceptibility. Further, women with *BRCA1* mutation have a 40–60% risk of ovarian cancer, while women with *BRCA2* mutation have a 15–20% risk. Men with BRCA2 mutation have a 5–10% lifetime risk of breast cancer.

Muir-Torre Syndrome and Other Rare Syndromes

Muir-Torre syndrome (MTS) comprises an association of skin tumors and adenocarcinomas of the gastrointestinal and genitourinary tract. The risk of breast cancer in woman with MTS is 12%. It has been hypothesized that MTS is a variant of HNPCC. Patients from two families with MTS have been found to have tight linkage to the mismatch repair gene *MSH2*.[15] Cowden's disease is a rare autosomal dominant condition that includes many different tumors and bilateral breast cancer. Breast cancer in this condition occurs at a young age (<20 years). The exact genetic change has not been identified. Peutz-Jeghers syndrome (PJS), which is also inherited as an autosomal dominant condition, is identified clinically by mucocutaneous pigmentation and hamartomatous polyps of the gastrointestinal tract. Breast cancer in PJS is usually bilateral and occurs between the ages of 20 and 40 years. The responsible genetic abnormality is unknown.

Prophylactic Mastectomy

Bilateral prophylactic mastectomy involves complete removal of all the breast tissue in the absence of any documented malignancy. This major surgical procedure may be offered to women who are at high risk for the development of breast cancer based on the detection of *BRCA1* or *BRCA2* mutations, or the recognition of Cowden's disease or PJS. However, there are no convincing data to demonstrate that the mortality associated with breast cancer in *BRCA1* or *BRCA2* individuals will be reduced by performing prophylactic mastectomy. With the recent significant advances in the detection of hereditary breast cancer, it is likely that increasing numbers of patients will be identified who are at high risk for the development of breast cancer. Prophylactic mastectomy may be a reasonable option for these individuals. Long-term prospective studies will be needed to better define the optimal management. The true incidence of breast cancer in individuals with *BRCA1* and 2 mutations who have undergone prophylactic mastectomy will need to be determined as well as the psychological impact of this procedure.

SUMMARY

The three known mechanisms of cellular transformation and oncogenesis include mutations in proto-oncogenes, inactivation of both copies of a tumor suppressor gene, and defects in DNA mismatch repair genes. Examples of each are included to substantiate the importance of understanding these mechanisms. *RET* is a proto-oncogene that is fundamental to the pathogenesis, and in the current era, molecular diagnosis of MEN 2 syndromes. *TP53* is a tumor suppressor gene that is mutated in individuals with Li-Fraumeni syndrome. *CDKN2* is a tumor suppressor gene that is mutated in pancreatic cancers and is associated with a poorer prognosis and the development of melanoma. *MSH2* is a mismatch repair gene that is important in the pathogenesis of HNPCC and Muir-Torre syndrome. Altered gene function such as loss of *DCC* in colon cancers may affect cell adhesion properties and promote metastases. As we begin to better define and understand the mechanisms of neoplasia, we will be able to improve current diagnosis and treatment.

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