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CANCER MECHANISMS

Multiple Endocrine Neoplasia Type 1 (MEN1) as a Cancer Predisposition Syndrome: Clues into the Mechanisms of MEN1-related Carcinogenesis

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Cancer is a major public health problem in the United States and around the world with millions of new cases diagnosed each year. While most of the cancers occur sporadically, a number of familial cancer predisposition syndromes have been identified in recent years. Cancer susceptibility syndromes are responsible for an estimated 5 to 10 percent of all cancer cases. Genes responsible for hereditary cancers are often also mutated in sporadic cancers. Studies of cellular functions of cancer susceptibility genes have made an invaluable contribution to our understanding of tumorigenesis.

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant cancer predisposition syndrome, the gene for which encodes a nuclear protein: menin. The biochemical function of this protein is largely unknown, but several studies have shown a role in transcriptional modulation through interaction with histone deacetylases and histone methyltransferases. Tumors in MEN1 arise through a two-hit mechanism, suggesting that menin either negatively regulates cell growth or participates in maintenance of genomic integrity.

In this work, we provide a general overview of the hereditary cancer syndromes with both dominant and recessive mode of inheritance. We then discuss recent advances in understanding the biological role of *MEN1* gene and the mechanisms by which inactivation of MEN1 may lead to neoplastic transformation.

HEREDITARY CANCER PREDISPOSITION SYNDROMES

Despite the complexity of cancer as a disease, there are some common features shared by all cancer cells that distinguish them from their normal counterparts. They are immortal and have a high proliferative potential. They no longer require external growth signals to activate the proliferation

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†Abbreviations: MEN1, multiple endocrine neoplasia type 1; RB1,retinoblastoma; LOH, loss of heterozygosity; DEB, diepoxybutane; MEFs, mouse embryonic fibroblasts; RPA, replication protein A; ES, embryonic stem.

program and are insensitive to growth inhibitory signals. They have developed mechanisms for evading senescence and programmed cell death, or apoptosis. In order to ensure tumor growth, the cells of solid tumors must promote the formation of new blood vessels for oxygen and nutrient supply. And, finally, malignant cells fail to exhibit normal cell-cell interactions, which results in their ability to invade and to metastasize and grow in an abnormal cellular environment [1]. The acquisition of these qualities is not a single event but rather the result of an accumulation of multiple genetic alterations leading to escape from normal control of growth regulation and differentiation [2].

Genes with very diverse cellular functions have been implicated in carcinogenesis. This includes genes involved in intracellular signaling cascades, cell cycle control, cell differentiation, apoptosis, protein synthesis and degradation, organization of cytoskeleton, cell migration, cell-cell interaction, regulation of transcription, chromatin remodeling, and DNA damage sensing and repair [3-5]. Understanding how the disruption of these cellular processes leads to tumor formation is the key to rational development of effective cancer therapies.

Because the development of cancer is a multi-step process, it may take years between the occurrence of the first genetic lesion and the actual formation of malignant neoplasm. Thus, the incidence of cancer increases with age. In rare cases, however, individuals inherit a mutant allele of a cancer predisposition gene, which increases the mutation rate and promotes the development of cancer. Thus, individuals with cancer predisposition syndromes have both a greater risk of developing cancer and an average earlier age of onset compared to the general population.

In 1971, Knudson proposed his famous "two-hit" hypothesis [6]. By observing patients with familial and sporadic retinoblastoma, he suggested that individuals with familial cases inherited the first genetic lesion, or "hit," and that they only needed to acquire one more hit to develop cancer. These patients developed retinoblastoma at an earlier age than individuals with sporadic tumors who needed to acquire two somatic hits in the same cell. This hypothesis was confirmed with the discovery of the retinoblastoma (RB1) gene in 1986 [7]. Indeed, individuals with familial retinoblastoma inherited a germline mutation in one allele of the RB1 gene with the remaining wild type allele being lost in tumors, often through a process involving large deletions with loss of heterozygosity (LOH) for surrounding polymorphic loci [7].

Since the discovery of RB1, multiple tumor-predisposition genes have been identified by the study of familial cancer syndromes. It is estimated that 5 to 10 percent of all cancers develop in individuals who have inherited a mutation conferring cancer susceptibility [8]. Analysis of the genes responsible for hereditary cancer predisposition and the pathways in which they act has been critically important in providing insight into cancer formation, because the same genes that are affected in cancer-predisposition syndromes are often responsible for the majority of somatic cancer cases as well. For example, germline mutations in the APC gene account for less than one percent of all colorectal cancers, but APC is also inactivated in nearly all sporadic colon cancers [2].

More than 50 cancer predisposition syndromes have been identified to date [8, 9] with both dominant and recessive modes of inheritance (Table 1). Activating mutations in oncogenes occur only in a few cases, such as RET in multiple endocrine neoplasia type 2 (MEN2) [10] and MET in familial papillary renal carcinoma [11]. Activated oncogenes function dominantly in the cell, and mutation of the second allele is not required. Most of the dominantly inherited cancer syndromes, however, have an inactivating mutation in a tumor suppressor, and disease formation follows the two-hit rule. The first hit is a germline mutation that exists in every cell of the body. Somatic inactivation of the second allele of the same locus in certain tissues may initiate neoplastic transformation. Thus, cancer predisposition syndromes often have dominant mode

of inheritance at the level of the organism, but at the cellular level they are recessive because inactivation of both copies of the gene is required for cancer formation.

Genes whose inactivation leads to tumorigenesis are divided into two distinct groups: gatekeepers and caretakers. Gatekeepers are usually "classical" tumor suppressors that directly regulate cell growth, differentiation, and death in certain tissues. Dysregulation of a gatekeeper is necessary to initiate neoplastic transformation. Examples of gatekeepers include APC for colon epithelia, RB1 for retinal epithelia, NF1 for Schwann cells, and VHL for kidney cells [2, 12]. Inactivation of both homologs of these genes is found both in hereditary forms of cancer and in the majority of sporadic cancers of the same type. In contrast to gatekeepers, caretaker genes do not directly regulate cell growth. Caretaker genes are involved in DNA repair and maintenance of genomic integrity. Inactivation of a caretaker does not directly promote tumor formation but facilitates the development of mutations in gatekeeper genes and other cancer-related genes. Caretakers, like gatekeepers, may be tissue-specific, but mutation in a caretaker is neither necessary nor sufficient for the development of cancer. Mutations in some caretaker genes cause human cancer by the two-hit mechanism. For example, patients with familial breast cancer syndromes inherit one mutant copy of BRCA1 or 2, and LOH for the second allele is found in tumors of breast and ovary [13-16]. Similarly, in hereditary non-polyposis colorectal cancer (HNPCC), one mutant copy of mismatch repair genes such as MLH1, MSH2, or MSH6 is inherited, and the wild type allele is lost in tumors of the colon [17]. However, unlike gatekeepers, mutations in caretakers are uncommon in sporadic tumors of the types seen in the hereditary disease [18].

A number of human cancer predisposition syndromes have a recessive mode of inheritance (Table 1). These syndromes are also called genetic instability syndromes, because one of the hallmarks of these diseases is chromosomal instability and hypersensitivity to various DNA damaging insults. The genes mutated in these diseases, such as ATM, Fanconi anemia complementation group, NBS1, Xeroderma pigmentosum-associated genes (XPA, XPC, etc.), BLM, and RECQL4, function in DNA damage sensing or repair [4,8,9] and can be classified as caretakers by analogy with the BRCA and HNPCC genes.

Since the identification of the first cancer predisposition gene (RB1) in 1986, tremendous advances have been made in understanding the function of multiple genes implicated in both sporadic and hereditary cancers. However, the mechanism of action of many more recently identified genes is not well understood. Further studies are necessary to provide new insights into human neoplasia and improve the diagnosis and treatment of both sporadic and hereditary cancers.

MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN1)

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant cancer predisposition syndrome characterized by parathyroid, pancreatic islet, and pituitary tumors as well as other neoplasms such as gastrinomas, carcinoids, lipomas, angiofibromas, angiomyolipomas and thyroid tumors [19]. Clinical manifestations depend on the affected organ. Most MEN1-associated tumors are not metastatic but create clinical effects by overproduction of hormones such as insulin, glucagon, prolactin, parathyroid hormone, gastrin, growth hormone, or adrenocorticotropic hormone [19, 20]. The most common manifestation of the disease is parathyroid adenoma, with penetrance reaching almost 100 percent by the age of 50 [19]. Major causes of morbidity and mortality in MEN1 are gastrinomas and foregut carcinoids, which are often highly metastatic [19]. The estimated prevalence of MEN1 is one in 30,000 [21].

MEN1 was localized to chromosome 11q13 by linkage analysis, and the gene was later identified by positional cloning [23, 24]. Tumors in MEN1 arise through the two-

Table 1. Familial cancer syndromes and cancer predisposition genes [8-9].

Cancer syndrome *Dominant mode of inheritance*	**Type of tumors**	**Gene(s)**	**Function**
Familial retinoblastoma	Retinoblastoma Osteosarcoma	RB	Regulator of cell proliferation
Li-Fraumeni syndrome	Soft tissue Osteosarcoma Breast cancer Leukemia	p53 CHK2 [68]	Transcription factor, DNA damage response Kinase, regulator of p53 activity
Gorlin syndrome	Basal cell carcinoma	PTCH	Transmembrane receptor for hedgehog
Familial adenomatous polyposis (FAP)	Colorectal cancer	APC	Regulation of ß-catenin
Hereditary non-polyposis colorectal cancer (HNPCC)	Colorectal cancer	MSH2, 6, MLH1, PMS1, 2	Mismatch repair
Neurofibromatosis type 1	Neurofibromas	NF1	GAP for p21
Neurofibromatosis type 2	Schwannomas, Meningiomas	NF2	Links membraine proteins to cytoskeleton
Familial breast/ovarian cancer syndrome	Breast cancer Ovarian cancer	BRCA1/2	DNA repair
von Hippel-Lindau syndrome	Renal cancer (clear cell)	VHL	Transcription elongation factor
Hereditary papillary renal cancer (HPRC)	Renal cancer (papillary)	MET	Transmembrane receptor for HGF
Familial melanoma	Melanoma	p16	Cyclin-dependent kinases CDK4 and CDK 6 inhibitor
Multiple endocrine neoplasia type 1 (MEN1)	Pituitary Parathyroid adenoma Pancreatic islet	MEN1	Transcriptional regulator, modulator of AP-1 activity
Multiple endocrine neoplasia type 2 (MEN2)	Medullary thyroid cancer	RET	Transmembrane receptor
Cowden disease	Breast cancer Thyroid cancer	PTEN	PI3kinase/AKT inhibitor, phosphatase
Tuberous sclerosis	Rabdomyoma Renal cancer	TSCI/2	Regulation of cell size and proliferation through insulin signaling

hit mechanism with germline mutation in one copy of the gene and somatic loss of the normal allele [22]. Germline mutations in *MEN1* are mainly nonsense or frameshift and are presumed to cause inactivation of the gene. The transcript of the *MEN1* gene contains an open reading frame predicted to encode 610 amino acid proteins. Analysis of the sequence of the protein menin showed two nuclear localization signals near the carboxyl terminus but has not provided any other clues to its function. Immunocytochemical studies of interphase somatic cells show that the protein is located in the nu-

Table 1. Familial cancer syndromes and cancer predisposition genes [8-9] (cont.).

Cancer syndrome *Recessive mode of inheritance*	Type of tumors	Gene(s)	Function
Bloom syndrome	Solid tumors	BLM	Helicase DNA repair
Ataxia telangiectasia (AT)	Lymphoma	ATM	DNA repair DNA damage response
Nijmegen breakage syndrome (NBS)	Lymphoma Glioma Medulloblastoma	NBS1	Double strand break repair
Xeroderma pigmentosum	Skin cancer	XPB, XPD, XPA	Nucleotide excision repair
Rothmund Thompson syndrome	Basal cell carcinoma Squamous cell sarcoma	RECQL4	Helicase
Werner syndrome	Oseeosarcoma Meningioma	WRN	Helicase
Fanconi anemia	Leukemia Squamous cancers	FANCD2, FANCA FANCB, C, FANCE F, FANCG, L	DNA repair, DNA damage response

Abbreviations: GAP – GTPase-activating protein; MSN – mut S homolog; MLH – mut L homolog; PMS – postmeiotic segregation; HGF – hepatocyte growth factor; BRCA – breast cancer associated; PTEN – phosphatase and tensin homolog.

cleus [25]. Studies in human and mouse show that *MEN1* is expressed widely in adult and fetal tissues with high levels in the CNS, lung, liver, kidney, testis, and fetal thymus [23,26-28].

Among the earliest studies of menin function was a yeast two-hybrid analysis showing interaction between menin and JunD, a component of the AP-1 transcription factor [29]. Studies in mammalian tissue culture systems showed that the N-terminal half of menin could inhibit JunD-mediated transcription. Menin did not bind to other AP-1 components such as c-Jun, JunB, or four members of the Fos family. Further investigation of menin's involvement in JNK and other MAP kinase signaling cascades revealed that overexpression of *MEN1* in some cell lines inhibited ERK-mediated phosphorylation of JunD and Elk1 and JNK-dependent phosphorylation of JunD and c-Jun [30]. In addition, overexpression of menin in CHO-IR cells suppressed insulin-induced activation of AP-1 and transcriptional induction of c-Fos [31]. Similarly, menin was shown to bind to and inhibit transcriptional activation by several members of the NF-κB family of transcription factors [32]. Binding of menin to SMAD3 appears to enhance transcriptional activation function, and loss of menin blocks TGF beta signaling [33]. The homeobox-containing protein Pem [34] has also been shown to bind menin in biochemical assays. These data suggest that menin may act as a transcriptional regulator. Our investigations of the *Drosophila MEN1* homolog showed *in vivo* interaction of *MEN1* with JNK signaling pathway components. We also found that when tethered to DNA, *Drosophila* menin acts as a transcriptional repressor. Menin does not have any recognizable DNA binding motifs, and data are conflicting as to whether menin can directly bind DNA and whether it binds in a sequence-specific manner [35,36].

Multiple lines of evidence exist to support menin's involvement in transcriptional control and chromatin modification. Human menin was found to bind and control the ac-

tivity of multiple transcription factors. Menin interacts with mSin3a, a component of the Sin3/Rpd3 HDAC complex [37], and with a histone methyltransferase complex containing the trithorax family proteins MLL2 and Ash2L [38-41]. In addition, menin was found to bind to the promoter regions of several homeobox genes and to regulate their expression [38,39]. Genome-wide analysis of genomic binding sites showed menin's localization to the promoters of multiple genes [42,43]. Recently, two other transcription targets of menin were identified. Cyclin-dependent kinase inhibitors $p27^{kip1}$ and $p18^{Ink4c}$ are downregulated in menin-deficient cells, which also exhibited increased proliferation [40]. Thus, menin may control cell proliferation through regulating transcription of genes involved in cell cycle control.

That tumors in MEN1 arise through two-hit mechanism suggests that menin may act in the regulation of cell growth or in the maintenance of genomic integrity, as discussed above. Several lines of evidence suggest that menin might participate in DNA damage sensing or repair. Peripheral blood lymphocytes from MEN1 patients have an elevated frequency of chromosomal abnormalities after exposure to the cross-linking agent diepoxybutane (DEB) [44]. Menin-deficient mouse embryonic fibroblasts (MEFs) are hypersensitive to DEB [45]. Menin interacts with FANCD2, a gene that underlies the genetic instability syndrome Fanconi anemia. Moreover, this interaction is strengthened after the exposure of cells to γ-irradiation [45]. In addition, menin was shown to interact with replication protein A (RPA) [46], a protein that binds single strand DNA and is important for several DNA repair pathways [47]. Studies from our laboratory show a role for the *Drosophila* homolog of *MEN1* in maintenance of genomic integrity. We found that inactivation of *Men1* in *Drosophila* results in flies that are sensitive to certain types of DNA damage, in particular to ionizing radiation and DNA cross-linking agents. In addition, *Men1*-deficient flies have an elevated mutation rate both at baseline and after treatment with ionizing radiation and nitrogen mustard (a cross-linking agent) [48]. Menin-deficient tissues from both *Drosophila* and mammals fail to undergo S-phase arrest in response to DNA damage by ionizing radiation [49]. These data suggest that tumor pathogenesis in the human disease may relate to genomic instability.

Studies also support a direct role for *MEN1* in the control of cell growth and differentiation. Overexpression of menin represses the proliferation and tumorigenesis of Ras-transformed NIH3T3 cells and insulinoma cells [50]. Data are conflicting as to whether menin-deficient mouse cells have increased proliferative capacity. One study found no cell-autonomous proliferative defects in $Men1^{-/-}$ embryonic stem (ES) cells or embryonic fibroblasts (MEF) [51]. However, another study showed that $Men1^{-/-}$ MEFs have increased proliferative capacity, and reintroduction of wild type menin suppresses their growth, possibly through an interaction with activator of S-phase kinase (ASK), a component of the Cdc7/ASK kinase complex [52]. Some phenotypes seen in mouse models of MEN1 suggest a direct role in growth control. For example, tissue-specific gene inactivation in pancreatic islet cells of the mouse leads to widespread hyperplasia, consistent with a similar role for the gene postnatally [53].

A mouse model with targeted disruption of the *Men1* gene showed that menin has an essential role in development [54]. The phenotype of heterozygotes was very similar to the human MEN1 phenotype with tumors of the parathyroid, pituitary, pancreatic islets, thyroid, and adrenal cortex. Homozygous mice died in utero. They appeared normal at E9.5 but by E11.5-12.5 were developmentally delayed and smaller than normal littermates. Virtually all fetuses were resorbed by E14.5. Morphological defects included an open neural tube, hypoplastic myocardium, and an extremely malformed liver with abnormal organization of the epithelial and hematopoietic compartments and increased apoptosis [51]. Conditional knockout of *Men1* in mouse pituitary and pancreatic islets showed that both these organs were

able to develop normally even if *Men1* was deleted in early embryogenesis. Pancreatic islets developed ß-cell hyperplasia and later, in six- to 12-month-old mice, pancreatic islet adenomas. Insulinomas and prolactinomas developed in some animals starting at nine months. The late tumorigenesis in these mice suggests that additional somatic events are necessary to promote cancer formation [53,55].

Two studies suggested a function of menin related to telomeres. Investigations in our laboratory [56] showed that menin localizes to telomeres during meiotic prophase in mouse spermatocytes. Menin foci were seen only at this location and not in other regions of meiotic cells. The foci were apparent as soon as axial elements began to form, and signals increased in intensity throughout prophase. However, menin did not co-localize with telomeric proteins, such as TRF2, in somatic cells at any stage of the cell cycle. Furthermore, telomeres of MEN1-related tumors were no different in length than telomeres of histologically similar sporadic tumors arising without *MEN1* mutations. Overexpression of menin did not influence telomerase activity as assessed by TRAP assays. Another study, which systematically examined the effects of tumor suppressor genes on telomere maintenance, suggested that loss of menin function might stimulate telomerase (hTERT) expression [57] and that menin might be involved in tumorigenesis through this mechanism.

Menin has also been found to associate with several cytoskeleton components. Menin's interaction with the intermediate filament proteins GFAP and vimentin was suggested to control its nuclear import [58]. Another cytoskeletal interactor of menin is nonmuscle myosin II-A heavy chain protein, which was found to co-localize with menin at the cleavage furrow during cytokinesis [59].

CONCLUSIONS AND OUTLOOK

In the 10 years since the *MEN1* gene was first cloned, a wealth of information concerning its possible function in tumorigenesis has been obtained. The accumulated genetic and biochemical data show that menin has multiple binding partners in a cell. By virtue of interacting with several transcription factors, menin is proposed to be involved in signaling pathways such as JNK, MAPK, TGF beta, and NF-κB; all of which have a well-documented role in cancer formation [60-63]. At the same time, through its role in chromatin modification, menin has been shown to affect the expression of c-Fos, hTERT, $p27^{kip1}$, and $p18^{Ink4c}$, which are also linked to neoplastic transformation [57,60,64,65]. On the cellular level, menin has been shown to regulate cell cycle progression and genomic integrity, both of which are often associated with a cancer phenotype.

The multiple functions of *MEN1* are not altogether unconnected. As a regulator of transcription, menin could control the expression of multiple factors involved in cell cycle regulation and the DNA damage response (see the examples above). The transcription regulation is likely achieved through menin's function in chromatin modification. At the same time, there is a wealth of evidence connecting the modification of chromatin with the maintenance of genome integrity [66]. Finally, the possibilities of MEN1's involvement in cell cycle regulation and genome maintenance are not mutually exclusive. MEN1 was shown to regulate proper S-phase arrest in response to exogenous DNA damage [49]. Similar regulation is necessary in the cell as endogenous DNA damage occurs every cell cycle during DNA replication [67].

Taken together, the accumulated knowledge of *MEN1's* potential functions and cellular partners suggests several possible mechanisms of its involvement in cancer formation. The key question is which of these functions or interactions play critical role in cancer development. One possible way to address this challenging problem is systematic investigation of tumors obtained from Men1 heterozygous mice as well as from MEN1 patients. One such study generated p18(-/-)Men1(+/-) and p27(-/-)Men1(+/-) mice and found that p18 functionally collaborates with

MEN1 in suppressing lung and neuroendocrine tumors [64,65]. Another important question is whether menin aids in the maintenance of genome integrity by regulating the cell cycle or by serving as a structural component of DNA repair machinery. To this end, analysis of mutational spectra could point to a DNA repair step(s) most deficient in cells lacking *MEN1*. Careful examination of interactions between menin and known DNA repair proteins is also necessary. Addressing these issues is of key importance for the understanding of *MEN1*-related carcinogenesis and for the development of effective therapeutic approaches.

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