MINIREVIEW

Mismatch Repair Genes and Microsatellite Instability as Molecular Markers for Gynecological Cancer Detection

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Due to major developments in genetics over the past decade, molecular biology tests are serving promising tools in early diagnosis and follow-up of cancer patients. Recent epidemiological studies revealed that the risk for each individual to develop cancer is closely linked to his/her own genetic potentialities. Some populations that are defective in DNA repair processes, for example in *Xeroderma pigmentosum* or in the Lynch syndrome, are particularly prone to cancer due to the accumulation of mutations within the genome. Such populations would benefit from the development of tests aimed at identifying people who are particularly at risk. Here, we review some data suggesting that the inactivation of mismatch repair is often found in endometrial cancer and we discuss molecular-based strategies that would help to identify the affected individuals in families with cases of glandular malignancies.

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Cancer is a leading cause of mortality in western countries and its incidence is linked for the most part to occupational exposure or living habits. Tremendous efforts have been made during the last decades to elucidate the basic mechanisms that lead a normal cell onto an oncogenic pathway and to design new strategies and protocols for curing this disease. Although some success has been obtained in the treatment of certain types of tumors, the results still remain limited. A major reason for that partial failure is that diagnosis usually occurs late in the biological history of the tumor: some 30 generations may be needed for the very first cell that starts a clonal expansion process to form a tumor mass that can be detected. During this silent growth phase, multiple genetic alterations affect the dividing cells that are progressively forming an individual tumor, which further acquire the ability to metastasize. In this context, prevention and early detection programs are thus considered efficient strategies in limiting the deleterious effects of the disease (1). Such approaches, i.e., detecting genome alterations or tumors at an early development stage, have already been tried using such techniques as detection of adducts within the DNA or tumor-specific markers in the body fluids or tissues (2). However, the success of these strategies is limited. The correlation between exposure, DNA adduct formation, and biological outcome is not straightforward enough to rationalize, the use of specific molecular markers is still restricted to a limited number of tumors, and detection occurs when the tumor is already developed. In contrast, the accumulation of mutations within the genome could be considered as a general and relevant molecular marker that would predict a higher risk to develop altered biological behaviors such as tumor development. Indeed, mutations are the end-points of several molecular events occurring in the cell, from exposure to xenobiotics and their metabolic activation, to DNA modi-

A database on mutations identified in HNPCC families is available at the following address: http://www.nfdht.nl

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fication, DNA repair, and mutagenic processes, integrating also the variability between individuals. Considering mutations as a significant molecular marker for estimation of the biological fate of a cell or a tissue, several groups have designed strategies and model experiments to detect mutations in a complex genome (3, 4) and, ultimately, to evaluate the relationship between the accumulation of mutations within a genome and the associated risk factor for developing cancer or other types of genetic diseases.

In this paper, we would like to present such a molecular approach designed for the early identification of women at risk for the development of gynecological cancers.

Origin and Fate of Mutations

Mutations in DNA have been implicated in degenerative processes such as aging, carcinogenesis, or genetic disorders (5–7). A small fraction of these mutations is believed to result from genuine replication errors, due to the intrinsic, although extremely low inaccuracy of replicative DNA polymerases duplicating undamaged DNA. Indeed, the fidelity of DNA replication relies on the cumulative effect of several steps such as nucleotide selection by the core polymerase, proofreading and excision of a misincorporated residue, enhanced accuracy mediated by some accessory proteins, and postreplication controls performed by a specialized repair process known as DNA mismatch repair. The largest cohort of mutations arises during replication as a consequence of damages in the parental DNA. Some of these damages may arise spontaneously, such as the deamination of bases or the loss of a base moiety, or as a result of normal metabolism. In this respect, the various active oxygen species represent a special risk for DNA integrity (8, 9). Finally, many DNA-damaging agents are present in the environment, occurring either naturally, such as UV light, or as a result of human occupational or recreational activity. These compounds, eventually after being metabolically activated, may form premutagenic lesions by reacting with DNA bases, the altered chemical structure of which often represents an efficient block to replicative DNA polymerase progression. To overcome the potential damaging effect of these lesions, organisms have developed several strategies, aiming either at eliminating these DNA damages by different types of repair processes, or at tolerating those that are still present at the replication fork during DNA synthesis (9, 10).

The tolerance of DNA lesions can be achieved through two different strategies: damage avoidance (DA) and translesion synthesis (TLS). The DA process tends to ensure replication of a damaged template while avoiding the polymerase to replicate through the lesion. This can be achieved by postreplication recombinational repair (11), or by a polymerase strand switch (12). In the first case, the replicating polymerase blocked at the lesion site dissociates from its DNA substrate and reinitiates replication downstream from the lesion, leaving a gap that is filled by a recombination process involving the sister chromatid. In the second process, the polymerase dissociates from the damaged parental DNA strand and uses the newly synthesized strand of the undamaged sister chromatid as a template. After synthesizing a patch encompassing the lesion position, the polymerase switches back to the initial template strand. Whatever the mechanism used, DA is intrinsically error free. On the contrary, TLS requires that a polymerase reads through the damaged base at the expense of replicational accuracy, and is thus error prone in essence.

During the last 2 years, the understanding of the molecular mechanisms of TLS has dramatically evolved with the identification of new DNA polymerases that present the ability to copy modified bases (13). The previous model, elaborated mostly from genetic data in *Escherichia coli* (14), suggested that a replication complex was able to read through a slightly blocking DNA damage, although with decreased kinetics and fidelity. However, when encountering a lesion that strongly impairs the fork progression, the replication machinery required some accessory proteins, encoded by genes belonging to the SOS system, to perform TLS (13). This model does not seem to be valid anymore, as all organisms from archaea and prokaryotes to eukaryotes, including human, possess a pool of new DNA polymerases, forming the new Y family of DNA polymerases (13, 15) that are susceptible to replace the normal replication complex stalled at a blocking lesion, and to perform TLS over a short patch of nucleotides. Nevertheless, the complete TLS process is far from being elucidated, and some important questions remain to be addressed: for example, how is the exchange of polymerases regulated at the blocked replication fork? Which partners do they interact with? What is their specificity, in terms of lesions and mutations? Does a given TLS process require one or several polymerases, as was demonstrated for the bypass *in vivo* of a single benzo(a)pyrene adduct (16)?

Mismatch Repair Genes Expression and Genetic Instability

Mismatches might be introduced in DNA by spontaneous deamination of a cytosine to form an uracyl residue, by incorporation of modified nucleotides such as 8-hydroxy-dGTP, or as a result of DNA recombination. However, the main pool of mismatches result from misincorporation by the DNA polymerase of a normal nucleotide, which forms a mispair with the template base. These mispairs are processed by a specific DNA repair system (mismatch repair, MMR), conserved from bacteria (17) to human (18, 19), which is able to correct either a single base pair mispair or deletion/insertion loops of various size, resulting from the slippage of the nascent strand, particularly in repeated sequences, to restore the initial DNA sequence. In humans, six MMR genes have been identified (hMSH2, hMSH3, hMSH6 [GTBP], hMLH1, hPMS2. and probably hPMS1); however, the detailed molecular mechanism of the repair

process has not yet been fully elucidated nor reconstituted *in vitro*. It is, however, considered to process at least through three essential steps: 1) recognition of the mismatch and assembly of the repair protein complex; 2) degradation of the DNA strand containing the misincorporated nucleotide(s); and 3) repair synthesis. hMSH2, hMSH3, and hMSH6 share homology with *E. coli* MutS, and associate in two possible complexes, hMutSα (hMSH2/hMSH6) that recognize either a single mismatch and +1 insertions/deletions loops (20–22) or hMutSβ (hMSH2/hMSH3) that binds to +1 or larger insertions/deletions (23–25). Recent studies indicate that all of these MutS-like proteins have a DNA-stimulated ATPase activity and that MutSα, upon nucleotide and magnesium binding, forms a sliding clamp that translocates along DNA (26–28). The repairosome then assembles, involving a *E. coli* MutL homolog complex, hMutLα, formed by the association of hMLH1 and hPMS2 (29), and other partners such as the exonuclease 1 (30), which has a $5' \rightarrow 3'$ exonuclease activity, the endo/exonuclease FEN1 (31), polymerase δ (32), proliferating cell nuclear antigen (PCNA) (33, 34), the single-strand binding protein replication protein A (RPA) (35), and probably replication factor C (RFC) (36).

Inactivation of some of these genes has a dramatic effect on genome stability (37) and results in an accumulation of mutations (20, 38–41), as illustrated in hereditary nonpolyposis colorectal cancer (HNPCC), which represents 6% of all colon cancers. Affected patients, who present an elevated risk of colon tumor, as well as other cancers of the gastrointestinal and urogenital tracts (42–44), inherited a defect in one of the MMR genes, essentially hMSH2, hMLH1, and hMSH6 (about 35%, 55%, and 7% of all mutations analyzed, respectively), although few cases of mutation in hPMS1 or hPMS2 were reported[1]. This strong bias probably results from the fact that hMSH2 or hMLH1 are functionally unique in each repair complex, whereas the partial redundancy between hMutSα and hMutSβ results in a mild mutator phenotype, if any, in cells with hMSH3 inactivation (45). Tumor cells, which have further acquired a second mutation that inactivates the wild-type allele of the inherited defective gene, display an hypermutable phenotype as revealed by the instability of microsatellite sequences (46–49). Microsatellites are tandem repeats of simple sequence motifs that occur abundantly and randomly in the human genome. Analysis of DNA extracted from various types of tumors revealed a variation in the electrophoretic pattern of sequences of microsatellites as compared with sequences amplified from normal tissues (50, 51). This microsatellite instability results from the elongation of slipped intermediates that form during the replication of repeated sequences, and the persistence of these replication errors (RER^+ phenotype) in the genome is attributed to a defect in mismatch repair process (52, 53). Consequently, microsatellite instability is considered as a landmark of HNPCC tumors. It is noteworthy that such an instability has also been observed in normal tissues of some HNPCC patients and correlates with a reduction or loss of mismatch repair (54). On the contrary, due to the partial functional redundancy exposed previously for the hMutSα and β complexes, $hMSH6^{-/-}$ cells present high mutation rates but almost no microsatellite instability (55). Finally, although the inactivation of hMLH1 occurs by intragenic mutations in HNPCC families, an epigenetic process, namely the hypermethylation of CpG island in the 5′ region of the gene, seems to be a more common process in sporadic cancers (56–58).

Endometrial Cancer and Defective Mismatch Repair

Endometrial cancer demonstrates three distinct features that support the idea that this malignancy shares biological properties with colorectal cancers (59). First, the histopathological data suggest that endometrial cancers evolve in a manner similar to colon cancers, starting from hyperplasias through adenoma referred to as benign tumor. Second, the sequential inactivation of several genes such as Ki-ras, p53, and deleted in colorectal cancer (DCC) in both colon and endometrial tumors suggests that the same oncogenic molecular pathway may be shared, at least partly, for adenocarcinoma formation in both tissues (60). Third, endometrial cancer often occurs in women with HNPCC (61) for whom the risk of developing endometrial cancer is about 40%, and 20% for their kindred. Indeed, patients with HNPCC and accompanying different cancers such as endometrial, ovarian, ureter, kidney, pancreas, small bowel, and stomach cancers are classified as having Lynch II Syndrome (61). Additionally, other studies suggest the possibility that endometrial cancer may have a hereditary basis (59), and consequently, some families may be predisposed to the development of endometrial cancer. As in HNPCC-linked colorectal tumors, microsatellite instability has also been observed for other cancers, including endometrial and ovarian cancers (62, 63), which incidence among gynecological cancers are about 40% and 6%, respectively. Microsatellite instability among endometrial cancers varies from 17% to 32% in sporadic cancers (62, 64–66) to more than 75% of those tumors associated with HNPCC (62, 63). Accordingly, mutations in MMR genes were reported in these type of cancers (67–71). Moreover, hypermethylation of the hMLH1 promoter region has been detected in sporadic endometrial cancer (72–74). Alternatively, as noted above, some cases of endometrial cancers linked to the inactivation of hMSH6 failed to present the RER^+ phenotype (55).

For ovarian cancers, microsatellite instability ranges is detected in 10%–20% of examined cases (75, 76) and in up to 50% of cell lines originating from this type of cancers (77). These observations suggest that the instability might be common to some hereditary as well as to sporadic human cancers like endometrial or ovarian tumors. Because somatic instability is uncommon in the normal population, it might serve as a marker for the identification of a mutator phenotype.

Contemporary Endometrial and Ovarian Cancer Detection Possibilities

Although endometrial cancer is the most frequent cancer among genital tract malignancies, ovarian cancer is less frequent and more difficult to detect. In the past, many attempts have been made to apply general tests to detect these cancers at an early stage. The clinical examination of patient is still the most important, and should be followed by additional adequate investigation techniques such as Doppler color ultrasonography, computerized tomography, or magnetic resonance imaging. However, these types of examinations are not available for general screening due to their high cost and disponibility. Molecular biology techniques like estrogen/progesterone receptor status, oncogene and/or tumor suppressor genes expression, DNA ploidy, and telomerase activity were used to predict patients outcome or the recurrence of the disease. Estrogen/progesterone receptor loss in endometrial cancer has a negative correlation with patient survival and could be an early event during endometrial carcinogenesis (78). The binding of progesterone to its receptor is known to have an inhibitory effect on cancer cells via c-myc and c-jun mRNA levels, and it provides the molecular basis for gestagen treatment for those patients who present progesterone receptors in cancer tissue (79).

Several studies have examined the significance of ras oncogene mutations and its protein expression in endometrial carcinogenesis (80–82). The incidence of the Ki-ras mutation differs geographically among endometrial cancer origin from the United States (10%) or Japan (37%) (83). Expression of the Ki-ras protein varies from 30% to 100%, depending on the tumor histology, grading, and clinical stage of the cancer (82). The Ki-ras oncogene activation seems to be a risk factor for endometrial cancer.

About 20%–40% of endometrial cancers bear activating mutations in β-catenin (CTNNB1) gene, resulting in nuclear accumulation of β-catenin and, consequently, in enhanced signaling through Tcf/Lef transcription factors (84, 85). Abundant accumulation of β-catenin in precancerous lesions (atypical hyperplasias) suggests that aberrances of this oncoprotein may be early events during neoplastic transitions of the endometrium (84).

Tumor suppressor genes are recessive at the cellular level, and expression of one copy of the gene is enough to ensure its function. For a neoplastic event to occur, both copies of a tumor suppressor gene must be inactivated by point mutation or the loss of a portion of chromosome (86). In endometrial cancer, the status of PTEN/MMAC1, p53, and DCC tumor suppressor genes have been widely investigated. PTEN/MMAC1 (phosphatase, tensin homolog/ mutated in multiple advanced cancers) gene is mutated in up to 83% cases of endometrial cancer (87). Mutations are more frequent in microsatellite-unstable than in microsatellite-stable as well as in endometrioid compared with nonendometrioid types of endometrial cancer (88). Interestingly, PTEN/MMAC1 mutations have also been detected in over 40% of precancerous lesions, indicating that loss of PTEN function occurs at early stages of endometrial carcinogenesis (87). In contrast, mutations of the p53 gene appear as a relatively late event in endometrial carcinogenesis, with over 30% of cancers expressing an inappropriate protein as determined by immunohistochemistry (80). For the DCC gene, a high rate of loss of heterozygosity was noted, which resulted in an altered DCC mRNA expression. However, no relationship to tumor histopathology or clinical stage was observed (88). Other tumor suppressor genes like breast-cancer-susceptibility-gene 1 (BRCA1) and adenomatous polyposis coli (APC) do not appear to be frequently mutated. However BRCA1 and APC are frequently mutated in glandular cancers of the breast and colon, respectively. Preliminary data indicate that the APC gene is mutated in some cases of endometrial cancer (89).

A cellular DNA aneuploidy is associated with about 20% of cases of endometrial cancer. However, its analysis does not seem to be informative for patients with disseminated cancers. Relatively helpful for early endometrial cancer detection could be the assessment of the cells in S-phase or the determination of Ki-67 nuclear antigen levels present in proliferating cells by immunohistochemistry.

Telomeres are specialized nucleoproteins localized at the ends of each eukaryotic chromosome. Telomeric DNA consists of short tandemly repeated G-rich sequences that are not subjected to DNA semiconservative replication and, consequently, are expected to decrease in length after each round of replication. This shortening has been linked to the biological process of cell aging (90). Telomerase, a specialized ribonucleoproteic DNA polymerase, uses its RNA component as a template to add telomeric repeats at the end of each chromosome. In humans, this activity is present only in germline cells and is deficient in somatic tissues. Howeve, it is present in immortalized cell lines and in a large majority of human cancers. For these reasons, the telomerase activity can be possibly used as a method for early detection of cancer (91).

Conclusion

The early detection of cancers is generally considered of major importance for successful therapy (a controversial discussion is presented in Ref. 92), and this belief has led to the development of molecular epidemiology, which results from the merging of classical epidemiology and molecular biology (93). Epidemiological studies are of major importance as they ideally could help in precisely defining the populations of interest, identifying the factors that trigger and/or modulate the cause of cancer, and eliminating potential confounding factors (94). However, due to the constant progress in the knowledge of molecular mechanisms of carcinogenesis, molecular epidemiological studies are becoming complex, and long-lasting projects (95) and the technology transfer from research into preventive or curative practice are not yet effective (94). Because the current

medical practice generally requires short-term analysis techniques, specific and sensitive methods like polymerase chain reaction (PCR) or immunohistochemistry have been used to detect the multiple biomarkers that have been recognized as hallmarks for cancer development (96–100). Although efficient, these tests do not fulfill the expectations of preventive policies because they are designed to detect tumors already established and not to differentiate between at-risk and safe individuals among limited populations.

Such specific populations could be specified among families of patients affected from colon, endometrium, or ovarian cancers, as part of them are inherited diseases. Aside from the family history, the molecular identification of this subpopulation can be conducted by analyzing, in the tumoral tissue, the microsatellite stability or the MMR proteins status by immunohistochemistry. This is important to check because depending on the MMR status of the tumor, the therapeutical approach might be different as MMR-deficient cells are more resistant to drugs (101–103) and might be refractory to chemotherapy. Moreover, it is important to check the patient's relatives for any defect in MMR and to evaluate the familial history to identify HNPCC or Lynch II syndrome families.

On the other hand, PCR and immunohistochemistry (IHC) are used to target genes, or portions of the genome (microsatellites) or gene products (MMR genes) that are either involved in the instability of the genome when they are deficient (MMR genes), or indicate an ongoing instability of the genome (MIN) or a heterozygote status of oncogenes or antioncogenes (APC and MMR). In this case, the analysis indicates a potentiality of a further development of a tumor, any prior clinical indication. In this sense, it is used as a prospective diagnostic and prevention tool and for identification of at-risk subgroups.

Thus, the introduction of molecular biology tests would be important to identify affected individuals in these families and to propose to them a closer medical survey and eventually primary preventive surgery and postoperative adjuvant therapy to decrease the risk of cancer incidence.

Such tests should be performed on easily obtainable biological material such as blood, smears, or small biopsies, and should be sensitive enough to differentiate between tissues homozygous or heterozygous for the MMR function. Although it was suggested that an heterozygous status could be identified (104) on the basis of the accumulation of errors in DNA, another work failed to detect any difference between normal and MMR-defective cells, but the test used was probably not sensitive enough (105). For this last purpose, we believe that the mismatch amplification mutation assay (MAMA-PCR) (106) may have the required sensitivity. The MAMA-PCR procedure uses a specific primer specifically designed to detect a given mutation so that its last 3′-terminal nucleotide pairs with the mutated base (base substitution) or the 5′ adjacent nucleotide of the deleted base(s) (frameshift) on the template DNA, while its penultimate 3′ nucleotide form a mismatch with the template sequence (106). The PCR assay is based on the observation that this specific primer, which forms a 3′ terminal double mismatch when paired with a wild-type sequence, is amplified less efficiently by a DNA polymerase than when it pairs with the mutated sequence, where it forms a single mismatch at the penultimate 3′ position (106). The use of two instead of one 3′ terminal mismatches, as in usual allele-specific PCR, allows the sensitivity of the detection to be increased by two to three orders of magnitude (13). This method displayed a relative sensitivity of 10-5 in detecting carcinogen-induced mutations within a mutational hot spot sequence (4). Thus, such sequences appear to be exquisite molecular probes for assessing the accumulation of mutations. This assay has revealed, for example, that the APC gene is mutated in some cases of endometrial cancer (89).

These tests will allow the identification of affected individuals in families with cases of female genital tract malignancy. Within the family, we would be able to identify those individuals who are not at risk and do not need further medical surveillance and those who are at high risk and who will need further intensive care. Because the Lynch II families express not only gynecological cancers, but also colon, ureter, kidney, pancreas, small bowel, and stomach cancers, tests for gynecological tract malignancies will improve cancer detection rate in patients carrying germline mutations. Studies are currently in progress to validate this approach. The availability of easy-to-use, molecular biology-based tests for the identification of individuals at risk for developing cancer will be extremely important for gynecology, gastroenterology, surgery, and urology.

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