

Molecular Profiling of Gynaecologic Malignancies: A Review

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Abstract

The current pathological methods and existing serum tumor markers for gynaecologic malignancies does not provide efficient information regarding the therapeutic intervention to which a cancer responds. Cancers classified based on the molecular profile determines the abnormalities at the genetic level. This in turn has led to the discovery of cancer-specific biomarkers. The cancer-specific biomarkers are used against target specific gene products or pathways. A molecular profile includes both genetic and epigenetic signatures which are specific to a particular type of cancer. Genetic and epigenetic markers are more sensitive and specific which may be efficient in early diagnosis of cancer and aids in multiple therapeutic alternatives. In future, a combination of various biomarkers from genomics (genes, micro RNA [miRNA], mutations, Single Nucleotide Polymorphisms [SNPs]), proteomics (peptides, proteins, modifications) and metabolomics (small-molecule intermediates, hormones, systemic compounds) will provide a promising assay platform that suits specific treatment options.

Keywords: Epigenetics; Gynaecologic malignancy; Mutations; Single nucleotide polymorphism; Serum tumor markers

Introduction

Cancer is a complex genetic and an epigenetic disease driven by various endogenous and exogenous agents. Cancer ranks second in the death toll in the world followed after myocardial infarction. By 2020, the predicted cancer cases may reach 20 million with a death rate increasing to 12 million [1]. Unhealthy lifestyles (cigarette smoking, consumption of alcohol), adoption of the modern diet (high fat, low fibre content) and old age are among the main causes for increase in the cancer incidence. Cancer is a result of clonal evolution leading to a detectable premalignant lesion that may develop into a full-blown malignancy. Metastasis to vital organs is one of the primary causes for patient death. Surgery, followed by a combination of anti-cancer drugs (chemotherapeutic agents) with or without targeted radiotherapy is the standard treatment protocol for cancer. Most chemotherapeutic agents interfere with DNA synthesis, thereby halting the replication process in turn cell differentiation and proliferation. The new chemotherapeutic drugs target the cell cycle, growth factors and their receptors, signal transduction pathways, DNA repair mechanism, apoptosis and angiogenesis which are the hallmarks of tumorigenesis. These pathways may be affected either by genetic or epigenetic mechanisms that predispose to cancer.

Cancer, A Genetic Disease

Cancer is a well justified genetic disease caused by altered expression of genes by accumulation of mutations. Mutations can be caused by endogenous causes such as replication errors, chemical instability of certain bases (tautomerization) or by exogenous agents like free radicals, ionizing radiation, UV radiation, chemical carcinogens. Proto-oncogenes are cellular genes that are involved in growth and development. A mutated proto-oncogene is an oncogene that is permanently turned on and activated leading to cancer.

Oncogenes are generally activated by chromosome rearrangements and gene duplication. Tumor Suppressor Genes (TSGs) are normal genes that slow down cell division, repair DNA and signal cells to apoptosis and hence are involved in maintaining the integrity of the cell. Mutations in TSGs lead to uncontrolled cell division, a predisposing factor for the formation of cancerous lesion. Knudson proposed the 'two-hit' hypothesis that suggested that the transformation of normal cell into a malignant cell requires two discrete "hits" or molecular events in both alleles of a gene (TSG) involved in the control of cell proliferation leading to fully invasive cancer [2].

Gene mutations

Mutations are abnormal changes in the DNA that alters the genetic makeup of an organism. Even a single base change (point mutation) can also have a major effect. Some mutations may stop protein synthesis (non-sense mutations), while others may change the composition of protein and makes it inactive (insertion, deletion) leading to a disease. Some mutations may cause permanent activation of gene leading to amplified protein production (gene amplification) and few mutations may not have a noticeable effect (silent mutation). Accumulation of mutation is a preceding factor for cancer. Most of the mutations leading to cancer formation are inherited than acquired. Cancers that are inherited tend to occur earlier in life than acquired cancers. Acquired cancers mainly occur due to prolonged exposure to exogenous agents.

Mutations occur on a day to day basis and accumulate over a period of time if not rectified. An efficient DNA repair mechanism identifies such errors and corrects them. If the errors cannot be repaired, the cells are signalled to apoptosis. An inherited mutation which has a higher effect on gene function leading to a noticeable problem is called high penetrance mutation. High-penetrance mutations in cancer susceptibility genes or TSGs can lead to many people in a family getting similar kind of cancers-a family cancer syndrome. For example 1/5th of the breast cancer running in families is caused by high-

penetrance mutation of BRCA1 and BRCA2 genes. Some inherited mutations that do not affect gene function and are known as low-penetrance mutations. These mutations can cause subtle effects on hormone levels, metabolism, or other things that interact with the risk factors for cancer.

Cancer, An Epigenetic Disease

Cancer is also an epigenetic disease- patterns of altered gene expression without altering the primary DNA sequence. These alterations occur extensively in cancerous cells than normal cells. The epigenetic changes involve both loss and gain of DNA methylation (hypo and hypermethylation), as well as histone modifications and small, non-coding RNAs [3]. Hypermethylation leads to gene silencing, while hypomethylation results in increased transcription of genes. Identification of such genes may lead to the discovery of new biomarkers for the identification of tumor initiation and progression.

DNA methylation

DNA methylation is a covalent modification of the cytosine ring at the 5' position (5-methyl cytosine) of a Cytosine-phosphate-Guanine (CpG) dinucleotide. The methyl group is donated by S-adenosyl methionine (SAM) catalyzed by DNA Methyltransferases (DNMTs), present at the replication fork during the S-phase [4]. CpG dinucleotides are scattered throughout the genome but are highly concentrated in few areas referred as CpG Islands (CGIs) [5]. CGIs are densely present at the 5' promoter region of the genes. In normal cells, CpGs are scattered throughout the genome and are highly methylated, while promoter CGIs are scantily methylated [5]. DNA methylation at gene promoter CGIs leads to permanent expression silencing by direct inhibition of transcription factor binding to their relative sites by recruitment of Methyl-Binding Domain proteins (MBDs) [4].

The stable DNA methylation patterns alter as age proceeds as observed in cancer. DNA methylation is an alternative to mutations in silencing of TSGs. Global DNA hypomethylation was the first epigenetic alteration noted in cancer cells [6]. An average of 10% decrease in 5-methyl cytosine was observed in various cancers that affects both repetitive elements such as LINE1 and Alu and specific gene promoters. Hypomethylation results in genomic instability and reactivate normally silenced genes disrupting normal gene expression, activating growth-promoting and anti-apoptotic pathways [7]. Promoter hypomethylation also leads to reactivation of miRNAs resulting in silencing or aberrant expression of the corresponding protein [8].

Promoter DNA hypermethylation is frequently observed in many types of cancers. Evident promoter methylation was observed in mismatch repair (MMR) gene human mutL homolog 1 (MLH1) in colorectal cancer, DNA repair gene O-6-methyl guanine DNA-methyl transferase (MGMT) in gliomas and colorectal cancer and cell cycle regulator cyclin dependant kinase inhibitor 2A (CDKN2A/p16) in colorectal cancer and other malignancies to name a few [9]. DNA hypermethylation is known to be an early event in tumorigenesis that plays a role in tumor initiation and progression and provides a platform for the simultaneous accumulation of genetic and epigenetic aberrations. Genome methylation patterns can be used as biomarkers to assess tumour type, early detection and monitoring of prognosis, risk assessment and indicators of therapeutic response [10].

Histone modifications

DNA is wrapped around histone proteins to form nucleosomes which are comprised of a tetramer of two H2A and two H2B histone molecules flanked by H3 and H4 dimers. H3 and H4 histones have deacetylated positively charged N-terminal tails, which forms a closed and tight chromatin configuration around the negatively charged DNA. The addition of an acetyl group to the histones, loosens the tight bond between DNA and histones, resulting in an open chromatin configuration accessible for transcription machinery [11]. Two consecutive nucleosomes are linked together by linker histone H1. Post translational histone modifications affect the N-terminal tails and alter the DNA and histone binding. These modifications include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP ribosylation [11].

Acetylation and methylation of H3 and H4 histones are the most commonly studied histone modifications. Enzymes that catalyze these reactions are histone acetyltransferases (HAT), histone deacetylases (HDAC), histone methyltransferases (HMT) and histone demethylases (HDMT). These enzymes either activate or repress transcription depending on the specific substrate residue. The histone methyltransferase protein EZH2 catalyzes H3K27 trimethylation and its over expression was found to promote tumor growth in melanomas, lymphomas, prostate and breast cancers [12]. The chimeric oncoprotein promyelocytic leukemia-retinoic acid receptor β (PML-RAR β) produced by the (15:17) translocation in acute promyelocytic leukemia targets specific promoters by recruiting HDACs and HMTs, leading to silencing of gene expression [13]. DNA hypermethylation can also lead to aberrant HDAC and HMT recruitment to specific promoters [4].

Noncoding RNAs

Small noncoding RNAs are a family of RNAs that are complementary to the 3'untranslated region of mRNAs, leading to their degradation and subsequent inhibition of gene expression [14]. MicroRNAs (miRNAs), a part of this family is a 20-22 nucleotide synthesized first as long, noncoding RNAs (primary miRNA) processed by the RNA cleaving enzyme DROSHA to form a short hairpin RNAs (pre miRNA) in the nucleus. Pre miRNA is then transported into the cytoplasm and further cleaved by the enzyme DICER into double-stranded miRNAs [14]. miRNAs are then incorporated in the RNA-induced silencing complex and transported back to the nucleus, where they bind to complementary sequences of mRNAs and either degrades or silences the target mRNAs [14]. miRNAs are also epigenetically regulated at their promoter level and target many important TSGs. A single miRNA can have hundreds of target mRNAs.

miRNAs play a significant role in human neoplasia. let-7 family of miRNAs is aberrantly down-regulated in breast and lung tumours, leading to RAS pathway oncogenic activation [15]. Down-regulation of miR-15 and miR-16 was observed in chronic lymphocytic leukemia (CLL) and resultant activation of the BCL2 proto-oncogene [16]. Over expressed miRNAs include the miR-17-92 cluster, which plays a role in the development of lung cancers, breast cancers and CLL targeting the transcription factor E2F1, a major cell cycle regulator [17]. miR-17-92 cluster amplification is also observed in B-cell lymphoma [18].

Gene Variants/Single Nucleotide Polymorphisms (SNPs)

Many allelic copies of a single gene may be present but they are not mutations and such common differences are called variants. These variants are inherited and present in every cell of the body. A single base change known as single nucleotide polymorphisms (SNPs) is a most common variant. SNPs normally occur in non-coding regions and on an average of one SNP are present in every 300 base pairs across the genome (10 million SNPs in the human genome) [19]. They can act as biomarkers that help to locate genes associated with disease. If SNPs occur in a regulatory region of a gene, the gene's function may be altered and may result in a disease condition. Some SNPs may influence the function of genes in a subtle way by making them slightly more or less active [20]. SNPs may also be associated with phenotypic differences like drug resistance and propensity towards disease. SNP pattern is specific for each individual and hence a population can be grouped based on the SNP profile.

| Cancer | New cases | Death |
|-----------------|-----------|--------|
| Cervical cancer | 12,360 | 4,020 |
| Ovarian cancer | 21,980 | 14,270 |
| Uterine cancer | 52,630 | 8,590 |
| Vaginal cancer | 3,170 | 880 |
| Vulvar cancer | 4,850 | 1,030 |
| Breast cancer | 2,32,340 | 39,620 |

Table 1: Gynaecologic cancer statistics

Cervical cancer is the second most common cancer among women worldwide and the most common cancer in India affecting women between ages 30-55 [21]. Cervical cancer is caused by abnormal changes in either outer squamous cell lining or the glandular cells that secretes fluid during ovulation. Early vaccination given at the age of 11 or 12 years along with regular conventional Pap tests and HPV testing is now considered as the best way to prevent cervical cancer.

Ovarian cancer is the ninth most common cancer among women worldwide and second most cancer in women in India and is generally grouped with primary peritoneal and fallopian tube cancers [22]. About 85-90% of ovarian cancers are of epithelial origin. Currently, an effective screening technique is unavailable for early detection of the disease and hence diagnosed at advanced stages. The traditional methods of diagnosis include transvaginal ultrasonography and CA 125 blood tests which are nonspecific. High levels of CA 125 are also seen in non-cancerous conditions such as inflammatory conditions of the abdomen, recent surgery, fibroids, endometriosis, ectopic pregnancy or a ruptured cyst and thus alone cannot be used as an effective screening technique. If ovarian cancer is diagnosed early, the five-year survival may increase from 50% to above 90%. Ovarian cancer can also be hereditary due to mutations observed in high penetrance genes such as TP53, BRCA1 and BRCA2 genes.

Uterine cancer is the most common gynaecologic malignancy and cancer of the endometrium is the most common type of uterine cancer. Hormonal factors play a significant role in risk for endometrial cancer, with exposure to estrogen increasing the risk and exposure to

Gynaecologic Cancers

Gynaecologic cancers are cancers that begin in the female reproductive organs like cervix, uterus, ovaries, vagina and vulva. In this review, breast cancer has also been included as it is one of the most common cancers affecting female population. The gynaecologic cancers remain a black box as these cancers are usually diagnosed at late stages (stage III and IV). Uterine cancer is the most common type of cancer (approximately 52,500 new cases per year), while ovarian cancer is the deadliest due to late stage diagnosis. Cervical cancer is almost totally preventable given the availability of a Human Papilloma Virus (HPV) vaccine. The cancer statistics of the year 2014 for the gynaecologic malignancies including breast cancer is given in Table 1. (Cited from American cancer society 2014)

progesterone having a protective effect. A longer menstrual period in a women's life increases the risk for endometrial cancer, while use of birth control pills and pregnancy decreases the risk for endometrial cancer [23]. Endometrial cancer can also be caused due to mutations of rate limiting genes.

Primary Carcinoma of the Vagina (PCV) accounts for 1-2% of all gynaecologic malignancies and predominantly affects postmenopausal women [24]. 85-90% of vaginal malignancies are metastatic lesions. HPV-DNA has also been identified in PCV in only about 50% of the cases. PCV may develop either through HPV-induced mutation or through other non-HPV factors. Screening methods of vaginal cancer include routine pelvic examinations and Pap tests. Vaginal cancer can also be prevented by the HPV vaccinations.

Vulvar cancer is very rare and ranks 20th most common cancer in females and affects women above 50 years of age. Vulvar cancer is caused by the growth and spread of abnormal cells within the skin of the labia and perineum. Vulvar cancers are also linked to HPV infection, with about 50% of vulvar cancers thought to be caused by the virus [25]. Vulvar cancers are usually diagnosed in the early stages and are most often cured with surgical treatment.

Breast cancer can usually be detected during a screening examination. When cancer is suspected based on clinical breast examination or breast imaging (mammogram), microscopic analysis of breast tissue is necessary for a definitive diagnosis and to determine the extent of spread (in situ or invasive) and characterize the pattern of the disease. Classical treatment includes surgery combined either with

radiation therapy, chemotherapy, hormone therapy, and/or targeted therapy. About 5% - 10% of breast cancer results from inherited mutations of BRCA1 and BRCA2 genes [26].

Hereditary Gynaecologic Cancer

Around 5% of endometrial cancers, 10% of ovarian cancers and 5-10% of breast cancers are due to hereditary causes. Hereditary uterine and ovarian cancers are usually associated with Lynch syndrome. An attention to family history and ethnic background provides an insight in the diagnosis of hereditary cancers. The recognition of families predisposed to ovarian carcinomas in association with breast cancer is known as hereditary breast-ovarian cancer (HBOC) syndrome with BRCA1 and BRCA2 gene mutations [27]. The integral association of endometrial and ovarian carcinomas in families are attributed to Lynch syndrome II variant of Hereditary Nonpolyposis Colorectal Cancer (HNPCC) syndrome in which mutations of mismatch repair genes namely MSH2, MLH1, PMS2, MSH3 and MSH6 have been identified [28]. The genetic mutations can be identified by DNA testing and if the test is positive, the patients may be treated with specific targeted treatment and management strategies. If the test is negative, the conventional screening method can be followed.

Conventional Diagnostic and Prognostic Markers Used in Gynaecologic Cancer

Cancer is still a challenge, in spite of an extensive research and current interest in the field. The traditional pathological diagnosis may not be accurate as it is a subjective review of the cancerous tissue by a pathologist (dependent on the knowledge and experience) and hence may not be reproducible. It also provides little information about treatment regime. These methods lack the ability to detect the disease at an early stage. Serum biomarkers for early detection of gynaecologic cancers need to be identified and has become a primary priority. Different technologies are now used to localize the tumor, determine its stage, subtype and response to therapy which can be useful for early detection, diagnosis and treatment [29].

Current Serum Biomarkers for Gynaecologic Cancer

Cervical cancer

The Squamous Cell Carcinoma antigen (SCC) is the most commonly elevated serum marker, which makes up to 85–90% of all cervical carcinomas. Elevated pre-treatment serum SCC levels are related to the stage of the disease, size of the tumor, depth of the stromal invasion, the lymph-vascular space involvement and lymph node metastasis. Elevated SCC levels also have predictive value for prognosis [30]. The marker CYFRA 21-1 (serum fragments of cytokeratin 21) was found to be elevated in 42-52% of squamous cell carcinoma similar to the usefulness of SCC [31]. Along with SCC and CYFRA 21-1, CA 125, CA 19-9 and CEA were also elevated in cervical adenocarcinoma [32]. Another novel marker, Immunosuppressive Acidic Protein (IAP), was found to be elevated in both SCC and cervical adenocarcinoma [33]. The newer serum marker panel includes M-SCE, YKL-40, VEGF-C and Thymidine Kinase (TK) which are currently under investigation.

Ovarian cancer

Conventional Ultrasonography (USG) does not provide high sensitivity and unsatisfactory positive predictive values. Elevated serum CA 125 levels have been detected in 50% and 92% of ovarian cancers in early and late stages respectively. Changes in CA 125 levels correlate with the regression, stability and progression of the disease [34]. Elevated serum level of CA 19-9 was observed in 68–83% of mucinous ovarian cancers and only in 28–29% of non-mucinous types. Serum CA 15-3, CA 72-4 and CEA levels were also raised in ovarian cancer patients [35]. The serum markers for ovarian cancer that are under active investigation include Human Epididymis factor 4 (HE4) [displayed the highest sensitivity when compared to CA 125], serum Lysophosphatidic Acid (LPA) and sFas.

Endometrial cancer

Currently used serum markers include CA 125, which was found to be elevated in 11–43% of endometrial cancers. Pre-treatment CA 125 levels were shown to be related to the stage of the disease, the depth of myometrial invasion, peritoneal cytology and lymph node metastasis. Other serum markers which were found to be elevated include CA 19-9, CA 15-3, CA 72-4, CEA and IAP [36]. The novel serum markers include M-SCE, HE4 and human serum amyloid A (SAA).

Breast cancer

The American Society for Clinical Oncology (ASCO) recommended eight different protein-related tumor markers for breast cancer: CA 15–13, CA 27–29, CEA, Estrogen Receptor (ER), Progesterone Receptor (PR), human epidermal growth factor receptor 2 (HER2), urokinase Plasminogen Activator (uPA), and plasminogen activator inhibitor (PAI)-1. CA 15–13, CA 27–29 and CEA are biomarkers for monitoring; ER, PR and HER2 are markers for treatment planning; and uPA and PAI-1 are biomarkers for recurrence risk prediction. Other potential markers include p53, cathepsin D, cyclin E, and kallikrein 14 [37]. The novel panel of breast cancer markers include cytokeratins 8, 18, and 19, Kallikrein, osteopontin, mutp53 and crypto1 [38].

Need for Genetic Markers

A combination of biomarkers such as genes, proteins, miRNAs, SNPs and mutations derived from tissues, biofluids (serum, sputum, saliva, bronchial tear, CSF), and circulating tumor cells in the blood, bone marrow and nipple aspirate are needed to translate molecular signatures into clinical practice. Cancer cells are a carrier of genetic and epigenetic alterations leading to genetic instability and disturbed molecular pathways. When these changes manifest in majority of patients with a specific tumor type, they can be used as genetic biomarkers for detection, prognosis and targeted therapies. The development of high-throughput technologies such as next generation DNA sequencing, microarrays, mass spectrometry for gene and protein expression profiling has increased the rate of data acquisition for cancer. The molecular profile of an individual cancer is highly specific and allows the clinicians to determine the origin of the tumor, metastatic potential, drug responsiveness and recurrence. Molecular profiling of cancer overcomes the limitations of pathologic and serum markers. Anomalies are identified at genetic level leading to the discovery of cancer-specific targeted therapy [39]. Alterations in mitochondrial DNA (mtDNA) have also been suggested as biomarkers for numerous cancers [40].

Genetic Markers for Gynaecologic Cancer

Cervical cancer

HPV infection plays a major role in the pathogenesis of cervical cancer. HPV may function as a proto-oncogene in the invasive stages of the disease. The oncogene *myc* was found to be down regulated in cervical carcinoma, while *p16* was found to be over expressed in dysplasias and invasive cancer of the cervix [41,42]. Gene mutations in *MELK*, *ISG15*, *STAT1*, *IL 8*, *MMP1* and *MMP3* were found to play critical roles in the tumorigenic pathway and could be used as potential targets for newer therapies [43]. HPV genotyping and viral load is the most relevant test to identify specific oncogenic HPV infection and also for the stratification of cancer risk. The intensity of telomerase activity correlated with the severity of the abnormality in cervical biopsies and cytology [44].

It is also important to consider epigenetic changes in the viral genome and the host genome as well. Numerous reports demonstrate that TSGs have been silenced due to abnormal promoter hypermethylation in cervical carcinoma. The most common hypermethylated TSGs in cervical carcinoma is summarized in Table 2.

| Gene | Rate | Function |
|-------------|-----------|-----------------------------------|
| DcR1/DcR2 | 100% | Apoptosis |
| hTERT | 57% | Apoptosis |
| p73 | 39% | Apoptosis |
| p16 | 8-42% | Cell cycle |
| PTEN | 58% | WNT pathway |
| E-cadherin | 28-80% | WNT pathway |
| APC | 11-94% | WNT pathway |
| MGMT | 5-81% | DNA repair |
| FANCF | 30% | FA-BRCA1 pathway |
| BRCA1 | 6% | FA-BRCA1 pathway |
| MLH1 | 5% | Mismatch repair |
| RASSF1A | 0-45% | Negative Ras effector |
| DAPK | 45-100% | Metastasis/cell death |
| TSLC1 | 58-65% | Tumor suppressor |
| FHIT | 11-88% | DNA repair/Cell death |
| HIC1 | 18-45% | Transcription factor |
| RAR β | 33-66% | Cell differentiation |
| TIMP2/TIMP3 | 47%/1-10% | Tissue inhibitor matrix proteases |
| Caveolin-1 | 6% | Caveolae membrane |
| ER α | 25% | Steroid hormone receptor |

Table 2: Tumor suppressor genes hypermethylated in invasive cervical cancer.

Phosphorylated and acetylated forms of histone H3 shows a marked association with progression of the disease from CIN I to CIN II and CIN III [45]. The most common over expressed miRNA in cervical cancer include: miR- 199-s, miR-9, miR-199a*, miR-199a, miR-199b, miR-145, miR-133a, miR-133b, miR-214, and miR-127 and repressed miRNA include: miR-149 and miR-203 [46]. These observations demonstrate that both genetic and epigenetic pathways are the preceding factors during carcinogenesis of the cervix uteri.

Ovarian cancer

Several mutation studies have been reported for ovarian cancer that are associated with clinical outcomes. TP53 mutations are very common in serous carcinoma; KRAS mutations are prevalent in adenocarcinomas; CTNNB1 mutations are common in endometrioid carcinomas, but rare in serous, mucinous and Clear Cell Carcinoma (CCC); and PICK3CA mutations are most frequent in CCC. Different subtypes of ovarian cancer can be distinguished by specific gene mutations [47]. p53 is an independent marker for poor prognosis in ovarian cancer [47]. Mutations in KRAS, BRAF, PTEN, β -catenin (CTNNB1) and TGFBR2 genes have been reported in mucinous, endometrioid, and low-grade serous tumors and mutations in TP53, BRCA1, BRCA2, MLH1 and MSH2 genes have been reported in high-grade ovarian cancer subtypes [48]. Mutations in other genes such as RB1, NP1, FAT3, CS1, MD3, GABRA6 and CDK12 were also observed in ovarian cancer. Epidemiologic studies have clearly established the role of family history as an important risk factor for both breast and ovarian cancer. Germline mutations in the BRCA1/BRCA2 genes are associated with 15-40% lifetime risk of ovarian cancer. Nearly 2,000 distinct mutations and sequence variations in BRCA1 and BRCA2 have been identified.

It has become increasingly apparent that epigenetic events can also lead to cancer as frequently as mutations or Loss of Heterozygosity (LOH). Multiple genes are abnormally methylated in ovarian cancer that include p16, RAR- β , H-cadherin, GSTP1, MGMT, RASSF1A, leukotropon β 4receptor, MTHFR, PR, CDH1, IGSF4, BRCA1, TMS1, ER α , Km23, MLH1, MSH2 and others. The TSGs down regulated by both genetic and epigenetic mechanisms include PTEN, BRCA1, OPCMC, DIRAS/ARH1, PEG3, TES, MYO18B. TSGs that are down regulated by epigenetic changes alone include RASSF1A, DLEC1, ARL11/ARLTS1, p16, p21, MLH1, DAPK1, CDH1, FBOC 32, TGF and ANGPTL2 [49]. Gene expression also can be regulated at the post-transcriptional level by miRNA. The let-7/miR-98 families may play a role in both apoptosis and cell proliferation pathways and the miR-141/200 families were highly associated with Epithelial-to-Mesenchymal Transition (EMT) or chemosensitivity [50].

Endometrial cancer

Histological differences may be associated with distinct molecular genetic alterations like oncogenic activation and tumor suppressor inactivation that results in the development of endometrial carcinomas. The oncogenes that are commonly activated in endometrial cancer include the K-ras, B-raf, Her2/neu, β -catenin, AKT and FGFR2. The K-ras mutations are detected in approximately 10%–30% of endometrioid carcinomas and gain of K-ras function may be an early event in tumorigenesis and may also be associated with malignant progression of tumors [51]. B-raf mutations are relatively low in endometrial cancer and were correlated with decreased MLH1 expression [52]. Her2/neu over expression was also detected in about 10–20% of endometrioid carcinoma that characterizes late progression

and differentiation events [53]. β -catenin mutation results in the stabilization of proteins that are degradation resistant, that result in cytoplasmic and nuclear β -catenin accumulation. Nuclear β -catenin accumulation is seen more commonly in 31–47% endometrial hyperplasias than in endometrial carcinoma, suggesting a role in the early development of tumor [54]. Endometrial cancer is also known to possess various gene alterations which activate the PI3K-AKT pathway (reported 28% of cancers) and FGFR2 (observed in 10%) of primary uterine tumor samples [55,56]. PTEN mutations (25%–83% of tumours) are observed more frequently in endometrioid carcinomas with Microsatellite Instability (MSI) [57]. p53 mutation was found to be more frequent in tumours without hyperplasia (estrogen unrelated) than in those with hyperplasia (estrogen related). p53 mutations were observed in 90% of serous carcinomas and in about 17% of endometrioid type carcinomas [58]. DNA Mismatch Repair (MMR) deficiency with MSI, is a common molecular phenotype in endometrioid cancer and observed in 28% of sporadic cancers [59]. HNPCC patients with endometrial cancers have an inherited germline mutation in MLH1, MSH2, MSH6, or PMS2 genes.

Aberrant DNA hypermethylation has been reported to affect several genes in endometrial cancer. The MMR gene, MLH1 is typically silenced by DNA hypermethylation. In endometrial cancer, MLH1 promoter hypermethylation was seen in approximately 40% of the cases and was found to be an early event in tumorigenesis. Loss of MMR function genetically or epigenetically leads to MSI which accounts for 20-30% endometrial cancer cases [59]. Genes that are epigenetically silenced other than MLH1 include: APC, E-cadherin, CHFR, CASP8, TGF- β R2, p73, HOXA11, COMT (catechol-O-methyltransferase), MGMT among few [60].

Vaginal and vulvar cancer

Research is under progress to find new ways to prevent and treat cancer of the vagina and vulva. Vulvar cancer is more extensively studied than the vaginal cancer. There are two clinicopathological types of SCC of the vulva: those with HPV DNA and those without. These different clinicopathological features suggest that there may be different genetic changes in HPV-positive and negative cancers. The process of HPV-related carcinogenesis within SCC of the anogenital region depends on the viral E6 and E7 gene products. These genes bind with the p53 and Rb genes, respectively, inactivating their function in cell cycle regulation [61]. Gain of chromosome 3q was found to be a frequent finding in HPV-positive SCCs (50%), but not detected in HPV-negative cases and is an important oncogenic event in the progression of HPV-induced SCCs. Loss of the chromosome arms 11q and 3p were also frequently detected in vulvar cancers [62]. Recurrent gain of 8q was also detected but was found to be more frequent in HPV-negative (75%) compared to HPV-positive cancers (20%). 4p loss was observed only in HPV-positive cases [63].

Epigenetic alterations are distributed equally in HPV-positive and HPV-negative vulvar SCC. Very few reports have demonstrated the epigenetic aberrations in vulvar cancers. Genes like RASSF1A, RASSF2A, MGMT, p16 and TSP1 were found to be hypermethylated in vulvar cancer [64]. The silencing of RASSF genes (RASSF1A and RASSF2A) may provide cell growth advantage because of their suppressor functions such as activation of apoptosis, cell cycle control and microtubule stabilization. Methylated p16 promoter was associated with lymph node involvement at diagnosis, which may be used as a marker for tumor progression. TSP-1 hypermethylation was distributed equally in HPV-positive and HPV-negative tumours and

was associated with angiogenesis. MGMT hypermethylation was described as a late event in vulvar carcinogenesis and lack of expression is similar to p16 gene [64].

Breast cancer

Breast cancer is a complex and heterogeneous neoplasia with distinct pathologies, histological features and clinical outcome. The classification of subgroups based on gene expression profiling has altered the view of breast cancer profiling. The status of hormone receptors ER, PR and HER2/neu have been used as predictive markers for identifying a high-risk phenotype and for selection of the most efficient therapies [65]. 70% of the breast cancer cases have positive hormone receptors. Triple Negative Breast Cancer (TNBC) is the most aggressive subtype characterized by the lack of ER, PR, and HER-2 and designing a treatment for TNBC is currently a challenge [66].

Mutations also play an important role in instilling tumorigenesis in breast tissue. Alteration in TP53 gene was observed in 30% of breast cancer patients and varies in different breast cancer subgroups [67]. Approximately 80% of familial breast cancer cases are associated with mutations in BRCA1 and BRCA2 genes [68]. Family history profiles can predict BRCA1 or BRCA2 mutation. Germline mutations in other high penetrant genes are also attributed to hereditary breast cancers that include: TP53 mutations in Li-Fraumeni syndrome, STK11 (serine/threonine kinase 11) mutations in Peutz-Jeghers syndrome and PTEN (phosphatase and tensin homolog on chromosome ten) mutations in Cowden syndrome. Mutations in low-penetrating genes are also identified such as CHEK2 (checkpoint kinase 2), ATM (ataxia telangiectasia mutated), PALB2 (partner and localizer of BRCA2), and BRIP1 (BRCA1-interacting protein C-terminal helicase 1) [68].

Markers related to epigenetic changes in breast cancer are more useful for early diagnosis. Methylation of various genes such as cyclin D2, RAR β , Twist, GSTP1, p16, p14, RASSF1A and DAPK was observed in ductal lavage fluid and nipple aspirate of breast cancer patients [69]. Methylation of specific genes such as RASSF1A and APC was correlated with poor prognosis in breast cancer patients [70]. Hypermethylation of promoter regions of BRCA1 and BRCA2 genes are similarly affected as mutations in breast cancer. Hypermethylation of BRCA1 occurs in 10% of all sporadic breast cancers and increases the sensitivity to chemotherapeutic drugs and poly (ADP-ribose) polymerases (PARP) inhibitors [71]. TNBC tumours can be treated with the addition of DNA methyltransferase and histone deacetylase inhibitors. About 800 miRNAs have been identified in breast cancer patients. miR-375 and miR-122, exhibited strong correlation with clinical outcomes and metastatic relapse [72]. miR-122 acts as a tumor suppressor and plays an important role in inhibiting tumorigenesis through targeting IGF1R and regulating PI3K/Akt/mTOR/p70S6K pathway [72]. miR-497 was negatively correlated with pathological stage, lymphatic metastasis, tumor size, and HER-2 and no correlation with ER, PR and p53 status. Over expression of miR-497 results in downregulation of Bcl-w (antiapoptotic member of the Bcl-2 family), causing cellular growth inhibition and apoptotic enhancement. Elevated expression of miR-497 may thus have better prognosis, and can be used as a prognostic marker [73]. miRNAs can also modulate oncogenic or tumor suppressor pathways, including p53, c-MYC, RAS and BCR-ABL and hence may serve as novel diagnostic and prognostic candidates and potential therapeutic targets.

Genetic Variation/SNPs-an Important Genetic Marker

SNP patterns can be easily measured by linkage analysis and association studies that identify markers for genetic predisposition to disease [74]. SNP markers gives an insight into the genes that are involved in disease process and may serve as targets for therapies. SNPs are extremely stable over evolutionary time and unlikely to change over the lifetime of an individual. SNP data has the potential to provide more insight into genetic predisposition to cancer with least invasive techniques. The most commonly studied SNPs in gynaecological malignancies are given in the Table 3 (data retrieved from SNP database).

| Cancer | Chromosome | Gene | SNP | Risk alleles |
|-------------|------------|------------|------------|--------------|
| Cervix | 6 | CD83 | rs853360 | ---- |
| | | | rs9296925 | |
| | | | rs9370729 | |
| | | | rs9230 | |
| | | | rs750749 | |
| Ovary | B | SRD5A2 | rs523349 | CC, CG, GG |
| | 7 | PMS2 | rs7797466 | ---- |
| | 7 | XRCC2 | rs3218536 | AA, AG, GG |
| | 11 | PGR | rs1042838 | GG, GT, TT |
| | 11 | Intergenic | rs608995 | ---- |
| | 13 | RB1 | rs4151620 | CC, CG, GG |
| | | | rs2854344 | AA, AG, GG |
| | 13 | P2RY5 | rs2227311 | CC, CT, TT |
| Endometrium | 12 | MDM2 | rs2279744 | GG, GT, TT |
| | 22 | COMT | rs4633 | CC, CT, TT |
| | | | rs4680 | AA, AG, GG |
| Breast | B | CASP8 | rs17468277 | CC, CT, TT |
| | | | s1045485 | CC, CG, GG |
| | 6 | TNF | rs361525 | AA, AG, GG |
| | 6 | CDKN1A | rs3176336 | AA, AT, TT |
| | 7 | XRCC2 | rs3218536 | AA, AG, GG |
| | 8 | 8q24 | rs13281615 | AA, AG, GG |
| | 9 | CDKN2A | rs3731239 | CC, CT, TT |
| | 10 | FGFR2 | rs1219648 | AA, AG, GG |
| | | | rs2981582 | CC, CT, TT |
| | | | rs2420946 | CC, CT, TT |
| | | | rs2981582 | CC, CT, TT |
| 12 | CDKN1B | rs34330 | CC, CT, TT | |
| 13 | BRCA2 | rs1799954 | CC, CT, TT | |

| | | | |
|-----------|------------|------------|------------|
| 17 | BRCA1 | rs766173 | GG, GT, TT |
| | | rs144848 | GG, GT, TT |
| | | rs4987117 | CC, CT, TT |
| | | rs1799954 | CC, CT, TT |
| | | rs11571746 | CC, CT, TT |
| | | rs11571747 | AA, AC, CC |
| | | rs4987047 | AA, AT, TT |
| | | rs11571833 | AA, AT, TT |
| | | rs1801426 | AA, AG, GG |
| | | i4000379 | DD, DI, II |
| | | TP53 | BRCA1 |
| rs4986850 | AA, AG, GG | | |
| rs2227945 | AA, AG, GG | | |
| rs16942 | AA, AG, GG | | |
| rs1799966 | AA, AG, GG | | |
| i4000377 | DD, DI, II | | |
| i4000378 | DD, DI, II | | |
| rs1042522 | CC, CG, GG | | |

Table 3: Genetic variants of gynaecologic cancer.

Promising New Genetic Biomarkers Such as MammaPrint

A panel of genetic and epigenetic markers for diagnosis and prognosis of gynaecologic cancer is under research and one such breakthrough is MammaPrint. MammaPrint is the most promising diagnostic test to assess the risk that a breast tumor will metastasize to other parts of the body. This helps physicians to determine whether chemotherapy is beneficial for the patients or not. MammaPrint is based on the Amsterdam 70-gene breast cancer gene signature, which uses either paraffin embedded tissue or fresh tissue for the microarray analysis. MammaPrint is impeccable as it facilitates tailor made medicine based on individual profile. The 70-genes in the panel form highly interconnected networks and their expression levels are regulated by key tumorigenesis related genes such as TP53, RB1, MYC, JUN and CDKN2A. These are TSGs that are essential for tumor progression and metastasis and cover the six well-defined hallmarks of cancer, reflecting the acquired malignant characteristics of a cancer cell along with tumor progression and metastasis-related biological activities [75].

Conclusion

For gynaecologic malignancies, only a small handful of tumor-associated antigens are available as routine tumor markers. These markers try to serve as diagnostic tools, predictive prognostic marker and the clinical course after treatment but lack sensitivity and specificity. Biomarkers established using high throughput technologies such as proteomics and bioinformatics may provide more accurate

detection and management of gynaecologic cancers but currently also lack sensitivity and specificity to be individually used as a biomarker. Biomarker research is being actively carried out in developed countries and many effective markers are being presently used in clinical practice. In developing countries work on biomarkers is not so extensive and further research is definitely warranted for the establishment of more useful tumor markers.

Upcoming genomic and proteomic technologies are quite promising in identifying new biomarkers, which can significantly enhance the efficacy of cancer detection and management that may aid in individualizing the therapy in patients with specific molecular aberrations and also in monitoring therapeutic response. The new biomarkers change the rules of traditional screening and treatment procedures for gynaecologic cancers and allow the designing of rational intervention strategies. The future of cancer therapy lays in the identification of a biomarker which can predict the onset of cancer even before the development of cancer. Genetic markers will certainly help in efficient early diagnosis and provide appropriate direction in multiple therapeutic alternatives and hence definitely a promising option.

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