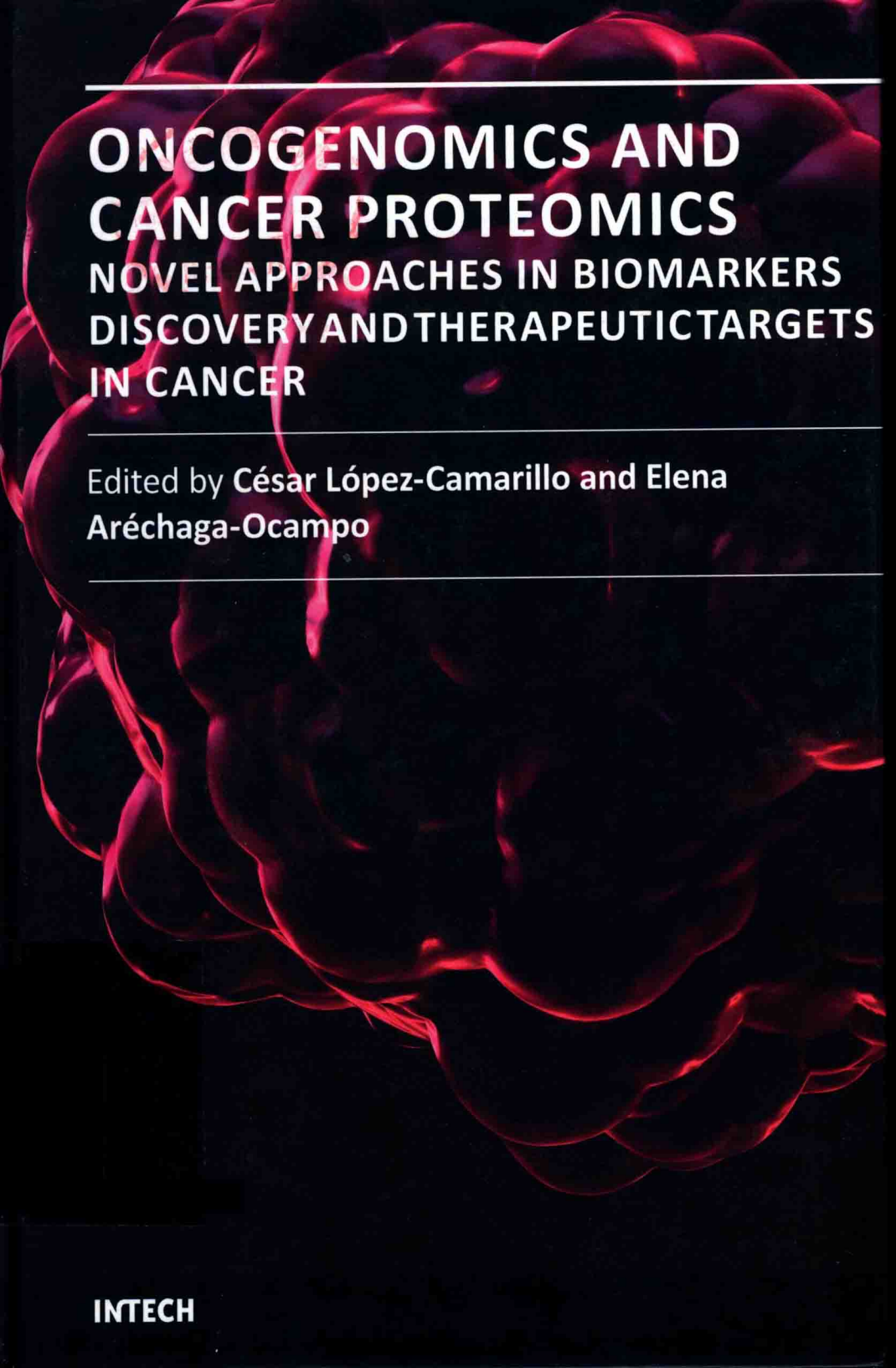

ONCOGENOMICS AND CANCER PROTEOMICS

NOVEL APPROACHES IN BIOMARKERS DISCOVERY AND THERAPEUTIC TARGETS IN CANCER

Edited by César López-Camarillo and Elena Aréchaga-Ocampo

INTECH

ONCOGENOMICS AND CANCER PROTEOMICS – NOVEL APPROACHES IN BIOMARKERS DISCOVERY AND THERAPEUTIC TARGETS IN CANCER

Edited by **César López-Camarillo**
and **Elena Aréchaga-Ocampo**

INTECHOPEN.COM

Oncogenomics and Cancer Proteomics – Novel Approaches in Biomarkers Discovery and Therapeutic Targets in Cancer
http://dx.doi.org/10.5772/1745
Edited by César López-Camarillo and Elena Aréchaga-Ocampo

Contributors
Norfilza M. Mokhtar, Nor Azian Murad, Then Sue Mian, Rahman Jamal, Elena Aréchaga-Ocampo, Nicolas Villegas-Sepulveda, Eduardo Lopez-Urrutia, Mayra Ramos-Suzarte, César López-Camarillo, Carlos Perez-Plasencia, Claudia H. Gonzalez-de la Rosa, Cesar Cortes-Gonzalez, Luis A. Herrera, Laurence A. Marchat, Elisa Azuara-Liceaga, Carlos Pérez-Plasencia, Lizeth Fuentes-Mera, Miguel A. Fonseca-Sánchez, Ali Flores-Pérez, Pouya Jamshidi, Clark C. Chen, Lili Jiang, Xueshan Qiu, Daniela Ferreira, Filomena Adega, Raquel Chaves, Mª Dolores Pastor, Ana Nogal, Sonia Molina-Pinelo, Luis Paz-Ares, Amancio Carnero, Hiroko Kozuka-Hata, Yumi Goto, Masaaki Oyama, Olga Villamar-Cruz, Luis E. Arias-Romero

Published by InTech
Janeza Trdine 9, 51000 Rijeka, Croatia

Publishing Process Manager Dimitri Jelovcan
Typesetting InTech Prepress, Novi Sad
Cover InTech Design Team

First published March, 2013
Printed in Croatia

Additional hard copies can be obtained from orders@intechopen.com

Oncogenomics and Cancer Proteomics –
Novel Approaches in Biomarkers Discovery and Therapeutic Targets in Cancer,
Edited by César López-Camarillo and Elena Aréchaga-Ocampo
p. cm.
ISBN 978-953-51-1041-5

INTECH
open science | open minds

Preface

Today, cancer research is focused on determining how genome and proteome level information may be useful as tools in prevention, diagnosis, and prognosis. The development of "omics" technologies, such as proteomics and transcriptomics has opened new research areas for scientists working on cancer research. This book presents the latest advances in cancer genomics and proteomics focused on identification of tumoral biomarkers and potential therapeutic targets in the most common human neoplasias including glioblastoma, oral squamous cell carcinoma, and breast, lung, prostate, and colorectal cancers. In addition, critical reviews of the relevant roles of microRNAs, animal models and the application of gene regulatory networks to validate potential therapeutic targets in cancer are also included.

Chapters in "Oncogenomics and Cancer Proteomics - Novel Approaches in Biomarkers Discovery and Therapeutic Targets in Cancer" present comprehensive and expert perspectives on the most common cancers from bench to bedside applications by an international team of experts in the field. This edited collection is subdivided into two sections titled: I) Genomic expression profiling in cancer, and II) Proteomic expression profiling in cancer. Proteomic technologies based on two-dimensional electrophoresis (2DPAGE and 2D-DIGE), or on isotope labeling methods followed by mass spectrometry (MS) analysis applied to the identification of differential protein expression in cancer are also discussed. This book will contribute greatly to the scientific and medical community by providing up-to-date discoveries of oncogenomics and their important roles in cancer translational research. It is intended for students, scientists, clinicians, oncologists and other health professionals working in the field of cancer research.

Dr. César López-Camarillo
Genomics Sciences Program, Autonomous University of Mexico City, Mexico

Dr. Elena Aréchaga-Ocampo
Cancer Biomedical Research Unit, National Institute of Cancerology, Mexico

Contents

Genomic Expression Profiling in Cancer

Genomic Expression Profiles: From Molecular Signatures to Clinical Oncology Translation

Norfilza M. Mokhtar, Nor Azian Murad, Then Sue Mian and Rahman Jamal

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53766

1. Introduction

Study related to diseases such as cancer has changed tremendously for a decade. For many years, the study was restricted largely to a single gene or a few genes in cancer cells. The studies have uncovered the roles of individual genes in the uncontrolled behavior of cancer cells. Studying the functional roles of genes in cancer cells has deepened our understanding not only the cancer cells as well as normal cells. Since 2003 onwards, the trend of publications was focusing on the analysis of thousands of genes with related molecular pathways. Steps taken from this analysis is then translated to clinical practice for the biological markers for an early detection, monitoring, prognosis of the disease and response to therapy.

The completion of the Human Genome Project in 2003 enabled a new era in biological sciences, in particular molecular medicine. The availability of the database of full sequences of approximately 3 billion base pairs and approximately 30,000 genes in human DNA will lead to a better understanding of physiological and pathophysiological changes in human body. Genome-wide expression technology allows the simultenous analysis of thousands of genes in a single experiment. The availability of the technology alters the way biological experiments can be designed. This has resulted of so called 'discovery biology'. The large amount of data produced by microarray resulted to new and unexpected features of cellular functions.

Since it was first introduced, microarrays are widely used for basic research, the development of prognostic tests, target discovery or toxicology researchs. The new form of cancer screening utilizes the molecular data generated from microarray studies. We will discuss the application of gene profiling data in the clinical screening of cancer. It is hopefully will give a broad picture the pipeline required to discover biomarkers of cancer.

The chapter is subdivided into a series of sections; each will discuss the scientific evidence on the molecular and cellular studies in selected cancers. We will try to critically assess the evidence upon which the theory on the cancer was built. The conversion of normal cells into cancer cells is a complex process and multistep processes. Scientists for many years tried to uncover the causes of cancer and emphasize certain oncogenes, or tumor suppressor genes or other groups of genes. Further information on how these findings were translated to the clinical settings will be provided. To date, with the massive gene expression profile data available to the researchers, there are still major hurdles in validating and reproducing the results. We will discuss the major drawbacks associated with the use of molecular signatures as the biomarkers or response to treatment.

2. Molecular signatures in colorectal carcinoma

Colorectal cancer (CRC) is a type of cancers that develops in the colon or the rectum of the human digestive system or gastrointestinal tract (1).Colorectal cancer is the third leading cause of death in both men and women in the US with 141,210 new cases and 49,380 death expected in 2011 (2). CRC progresses slowly over a period of time usually between 10 to 15 years (3, 4). The tumor begins with noncancerous polyps where the tissues that form the lining of the colon or rectum differentiate into cancerous tissues (5). Approximately, 96% of colorectal cancers are adenocarcinomas, which arise from the glandular tissue (6). It can grow along the lining of the epithelium into the wall of the colon and rectum and invade the digestive system (7). In addition, the cancerous cells can also penetrate into the circulating systems, the blood and lymphatic systems which known as metastasis (7). Typically, the cancerous cells will first spread into the nearby lymph nodes and subsequently penetrate into other organs such as liver, lungs and ovary through blood vessels (8, 9). Colorectal cancer can be classified as tumors/nodes/metastasis (TMN) staging and Dukes classification (12). The TMN assigns the number based on three categories, T, M and N, which are the degree of invasion of the intestinal wall, lymph node involvement and the degree of metastasis, respectively (10). The higher number of TNM system indicates the advanced stage of colorectal cancer (10).

Unhealthy lifestyles such as alcohol consumption, high intake of red meat, obesity, smoking and lack of physical activities are among the risk factors for CRC (1, 11). Age and gender also play significant role in the development of CRC as the risk is higher in male and elderly(7). People with inflammatory bowel disease such as ulcerative colitis and Crohn's disease are also at high risk of getting CRC (12). Among the patients with Crohn's disease, approximately, 2%, 8% and 18% of the patients will develop CRC after 10, 20 and 30 years, respectively (12). About 20% of patients with ulcerative colitis develop CRC within the first 10 years (13). Mutations in genes such as *KRAS, APC*, and *MMR* are the well-documented genetic factor that contributes to colorectal cancer (3, 14, 15). Individual with family history of CRC in two or more first degree relatives have 2 or 3-fold greater risk of getting CRC and this has accounted for 20% of all cases (7). Examples of CRC involving genetic mutations are hereditary nonpolyposis colorectal cancer (HNPCC or Lynch Syndrome), Gardner syndrome and Familial adenomatous polyposis (16).

Diagnosis of CRC is based on tumor biopsy performed during the sigmoidoscopy or colonoscopy (7). CT scan of chest, abdomen and pelvis could be performed to determine the metastasis state and in certain cases, PET or MRI may be used to assist in the diagnosis (7).Molecular testing for patients with a strong family history can be performed to identify mutation, thus initiate early diagnosis and screening in family members. In addition, molecular characterization of mutations involved in CRC may help doctors to plan a better treatment strategy for the patients. Managing our lifestyles can help us to reduce our risk of getting CRC, for example by improving lifestyle through regular exercise, increasing the consumption of whole grains, fruits and vegetables and reducing the red meat intake (17). The treatments for CRC include surgery, chemotherapy and radiotherapy.

2.1. Molecular biology of colorectal cancer

Colorectal cancer is a multistep process that includes accumulation of several genetic and epigenetic alterations (18, 19). It is well characterized that the adenoma to carcinoma sequence is due to accumulation of the genomic alteration, which is induced by genomic instability (4, 20). Genomic instability is an event, which will increase tendency of the genome to acquire mutations when several important processes in maintaining and replicating the genome are malfunction. It is a hallmark of many human cancers (20). There are three well-reported genomic instability pathways that could lead to colorectal cancer, which will be discussed in details below.

a. Chromosomal instability (CIN)

Chromosomal instability lead to increase rate of losing or gaining chromosomes during cell division and accounts for 15% to 20% of sporadic CRC as well as Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer) (21).There are three mechanisms involved in this process that includes structural chromosome instability, the chromosome breakage-fusion-bridge (BFB) cycles and numerical instability (22). Structural chromosome instability is caused by high incidences of DNA double-strand breaks, which may lead to abnormalities in chromosomal segregation during mitosis. Chromosomal damage may result in mitotically unstable chromosome, which may promote an event known as breakage-fusion-bridge (BFB) (22). An abnormal number of centrosome may be caused by abnormal mitotic polarity as well as unequal segregation of chromosomes during the anaphase stage (23). CIN promotes cancer progression by increasing clonal diversity (21). In the clinical perspective, large meta-analysis has shown that CIN is a marker of poor prognosis in colorectal cancer (20).

b. Microsatelite instability (MIN)

Microsatellites are repetitive sequences of DNA, which is highly varied between individuals (24). The most common microsatellites in human is a dinucleotide repeat of CA (25). MIN is a condition, which is manifested by damaged DNA due to defective in the DNA repair mechanism. CRC with the presence of MIN have a better prognosis compared to CRC with CIN (26). MIN involves the inactivation of the DNA Mismatch

Repair (MMR) genes via aberrant methylation or somatic mutation (26). HNPCC or Lynch Syndrome is an example of CRC, which is caused by MIN with 15% occurrence (27). MIN could cause CRC in 2 mechanisms; 1) mutations in the MMR genes where error in the microsatellite repeat replication is unfixed. This leads to the inactivation of tumor suppressor genes (TSG), a group of genes which is crucial in maintaining cell cycle progression and apoptosis induction (20). Inactivation of these genes may lead to tumorigenesis through uncontrolled cell division 2) epigenetic changes that silence the MMR genes (20).

c. **CpG Island Methylation and CpG Island Methylator Phenotype (CIMP)**

Hypermethylation of the promoter region of a gene that contains CpG Island (CGI) and global DNA hypomethylation are associated with epigenetic instability in colorectal cancer (20). CGIs are short sequences rich in the CpG dinucleotides and are observed in the 5′ region of almost half of all human genes (28). *In-vitro* study of BRAF in CRC cell lines showed no correlation between BRAF and CIMP (29).

2.2. Genome Wide Association Study (GWAS) in colorectal cancer

The completion of Human Genome Project in 2003 and the International HapMap Project in 2005 have opened up a new era in genetic and phenotype correlation study (30). The completion of these two projects has made the Genome wide association study (GWAS) possible. GWAS is considered as the most powerful tool to study the association between phenotypes and genotypes and also to identify common, low-penetrance susceptibility loci in a particular disease. In addition, GWAS can also be employed to investigate gene-environment interactions and the pooled analyses may also lead to the identification of novel modifying genes. Several GWAS studies have been performed in colorectal cancer and several loci were identified to be associated with CRC such as 8q24 (128.1-128.7 Mb, rs6983267) (31, 32). The *C-MYC (MYC)* oncogene is located approximately 300 kb from this region and is often over-expressed in CRC (33). Validation studies have confirmed that rs6983267 loci as the most promising variant in CRC, which has increased the chance of getting CRC by approximately 1.2 fold (33, 34). Recent publication has suggested that this variant is involved in enhancing the Wnt signaling and MYC regulation, which are known pathways in carcinogenesis (35). However, further functional analyses are still needed in order to determine the function of this variant. In the Japanese population, this variant leads to an increase risk of CRC with an allelic OR=1.22. Even after the adjustment for confounders, the OR remains significant (OR = 1.25). In the ARCTIC report, a locus at 9p24 was identified to be associated with CRC and was confirmed in the Colorectal Cancer Family Registry. Several numbers of loci that include 18q21:*SMAD7*; 15q13.3:*CRAC1*; 8q23.3: *E1F3H*; 14q22.2:*BMP4*; 16q22.1: *CDH1* and 19q13.1:*RHPN2* were also found to be associated with CRC. These genes have been shown to be involved in CRC progression. Studies conducted in Korean and Japanese patients with CRC have identified a novel susceptible locus in *SLC22A3*, which was significantly associated with distal colon cancer (36). The variant, rs7758229, was located on 6q26-q27 with OR=1.28. Three variants, rs7758229,

rs6983267 and rs4939827, in *SMAD7* together with alcohol consumption may increase the risk of CRC by approximately two-fold. Several variants including rs6983267, rs6695584, rs11986063, rs3087967, rs2059254 and rs72268855 showed evidence of association with CRC in Singaporean Chinese (31). sSNP rs3087967 at 11q23.1 was associated with increased risk of CRC in men (OR=1.34) compared to women (OR=1.07). The rs 10318 at locus 15q13 (GREM1) was also associated with CRC with OD =1.19 (37).

Almost half of the susceptibility loci in CRC are located nearby the transforming growth factor beta gene (*TGF-β1*), which is important in the carcinogenesis (38). An elevated level of *TGF-β1* was linked to tumor progression and recurrence in CRC. Germline mutations in components of *TGF-β1* signaling pathway such as SMAD4 is responsible for the high-penetrance juvenile polyposis syndrome. Other genes are *SMAD4, RHPN2, BMP4, BMP2* and *GREM1*.

2.3. Gene expression profiling in colorectal cancer

Gene expression profiling was performed to compare between colorectal adenomas and CRCs and the result showed that the level of six cancer-related gene sets were increased in CRCs compared to adenomas (FDR<0.05). These include genes that involved in chromosomal instability, proliferation, differentiation, angiogenesis, stroma activation and invasion. Changes in the activity of the chromosomal instability were the most significant gene set (FDR=0.004) (39). The key genes that are associated with colorectal adenoma to carcinoma progression are AURKA, TPX2 (Chromosomal instability), PLK1 (Proliferation), ADRM1 (Differentiation), SSCA1 (Stroma activation), SPARC and PDGFRB (Invasion). The expression levels of these genes were significantly higher in CRC compared to adenoma (p<1e-5). Overexpression of AURKA induces centrosome amplification, aneupploidy and cellular transformation *in vitro* (40). AURKA interacts with TPX2 and plays a role in centrosome maturation and spindle formation (41). The polo-like kinase 1 (PLK1) is important in spindle formation and cell cycle progression during the G2 and M phase (42).

Wu and colleagues showed that the extracellular matrix and metabolic pathways were activated and the genes related to cell homeostatsis were downregulated. In this study, they compared cancer transcriptome using massive parallel paired-end cDNA sequencing in 3 different tissues, CRC tissue (stage III), adjacent non-tumor tissue and normal tissue from a 57 years old female patient. They detected 1660, 1528 and 941 significant differential genes (DEGs) between the CRC and adjacent tissue, the CRC and normal tissue; and the adjacent and normal tissue respectively. 15-prostaglandin dehydrogenase (*15-PGDH*) was downregulated in cancer compared to normal tisssue, which is common oncogenic event in approximately 80% of CRC cases. The transition between adenoma and carcinoma processes involved inactivation of *TGFBR2*, thus progressive inactivation of this gene from cancer-adjacent and normal tissue was expected. In addition, *APC, MYH, CD133, IDH1* and *MINT2* were also dysregulated in CRC. They also identified many genes involved in extracellular matrix (ECM) receptor interactions were highly dysregulated in cancer. The findings showed that all collagen type proteins were overexpressed up to 1000-fold in cancer tissue.

In addition, members of MMP family, which degraded the ECM structures, were also induced significantly in tumor. These include MMP1, MMP3, MMP14 and MMP7. Other cell-cell adhesion-related molecules for examples laminins (LAMA4, LAMA5, LAMB1, LAMB2 and LAMC2) and integrins (ITGA5, ITGB5, ITGA11 and ITGBL1) were elevated in cancer tissues. It was suggested that "angiogenesis switch" was activated in tumor tissues since vascular endothelial growth factor (VEGF) was found to be upregulated. In conclusion, up-regulation of the ECM pathway and the angiogenic growth factors may lead to remodelling of the ECM pathways as well as expansion of the new vessel networks, which subsequently resulted in CRC progression. Since their results in concordance with previous studies that showed the ECM pathway was subjected to intensive epigenetic modification, therefore this ECM may be a good candidate as prognostic biomarkers in CRC (43).

3. Molecular signatures in ovarian cancer

Ovarian cancer is among the top ten leading cancers among women the United States. In this country alone, there are approximately 22,280 new cases and 15,500 estimated death in 2012 (44). At our local population, approximately 1627 women were diagnosed in 2003 to 2005 and the figure showed increasing trend in 2007(45).In Japan and Sweeden, the incidence of ovarian cancer per 100,000 women is 3.1 cases and 21 cases respectively (Green et al., 2012). Due to vague or absence of early signs and symptoms, patients suffer from this cancer seek late treatment (46). Therefore, the cancer is normally diagnosed late when the disease is not longer confined to the ovary. Based on different morphological characterisitcs of the cancer, it is divided into epithelial and nonepithelial types. The epithelial type is further subdivided into serous, mucinous, endometrioid and clear cells. On the other hand, the nonepithelial is granulosa cells, mixed germ cells tumour, immature teratoma, dysgerminoma and teratoma. The risk factor for this cancer is unclear, however the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study has recently documented that women who smoke more than 10 cigarettes a day had doubled the risk to develop mucinous ovarian cancer (47). This has suggested that the effect of smoking differs based on different histological subtypes of ovarian cancer(47). On the other hand, a study has shown that long period of breastfeeding seems to have reduced risk of ovarian cancer (OR = 0.986, 95% CI 0.978-0.994 per month of breastfeeding) (48).This effect of breastfeeding was also varies between histological subtypes as there was no association between breastfeeding and borderline serous or mucinous cancer (48).

Ovarian cancer was initially divided based on molecular pathways involved in the development and progression of the subtypes (49). Type I is low-grade serous, low-grade endometrioid, mucinous and clear cells. They are believed to arise from benign lesions such as ovarian inclusion cyst or endometriotic lesions. These lesions follow the stepwise pattern, whereby it evolved from the benign adenoma to borderline and finally to malignant tumours (table 1).

Type II ovarian cancer is high-grade serous, high-grade endometrioid and undifferentiated. The common mutations that are found in these subtypes are p53, BRCA1/2, PIK3CA with chromosomal instability. They normally involve the peritoneum and grow rapidly.

Characteristics of tumour	Type I	Type II
Type of tumor	Low-grade serous	High-grade serous
	Low-grade endometrioid	High-grade endometrioid
	Mucinous	Undifferentiated
	Clear cell	
Common mutations and genetic modifications	KRAS	p53
	BRAF	BRCA1
	PTEN	BRCA2
	CTNNB1	PIK3CA
	Microsatellite instability	Chromosome instability

Table 1. Ovarian subtypes based on common mutations and genetic modifications

In clinical practice, the gyneoncologist still use CA125 as the biomarker to monitor treatment of this cancer. However, it is not sensitive and specific to detect the cancer in its early stage (46). It is of great demand to find new molecular marker for the ovarian cancer.

Ovarian cancer is treated by surgery, radiation or platinum-taxane based chemotherapy depending on the subtypes and extent of the cancer (50). Patients at stage I and II will undergo bilateral salphingo-oophorectomy. While for advanced cases, adjuvant chemotherapy combined with surgery is highly recommended. With the latest understanding on the mutational types of ovarian cancer, mitogen activated protein kinase (MEK) inhibitor such as CI-1040 was used to test the potential therapeutic agent in *in vitro* ovarian cancer cell line (51). This cell lines containing KRAS or BRAF mutations, which are known mutations for type I ovarian cancer. The targeted therapy for type II ovarian cancer encounters difficulty due to lack of common molecular pathways. In two cohort studies involving 16 international centers, women with BRCA1 or BRCA2 mutation were treated with two different doses of Olaparib (52). This drug is orally active poly(ADP-ribose) polymerase (PARP) inhibitor. The result showed a promising therapeutic indexin ovarian cancer patients with mutation of BRCA1 or BRCA2 (52).Based on this study, Olaparib has possible as therapeutic agent in type II ovarian cancer.

3.1. Molecular biology of ovarian cancer

Ovarian cancer is a heterogenous disease and thus, there is no clear molecular genetics involved in the transition of normal ovarian epithelial cells into cancer cells. Approximately 10 to 15% of ovarian cancer is thought to run in the families (53). It is closely related to BRCA1 and BRCA2 mutation (53). It was recently published that suggested screening of BRCA1/2 mutation in patients with ovarian cancer prior to chemotherapy treatment (54). This is because presence of such mutations may influence the treatment outcomes (54). Human DNA repair mismatch genes for example MLH1 and MSH2 accounts for 10% of patients with hereditary nonpolyposis colon cancer syndrome (55). Other related genes include glutathione S-transferase M1 (GSTM1) is associated with endometrioid or clear cells ovarian cancer.

Approximately 85% of ovarian cancer is regard as sporadic with no apparent hereditary factors. Accumulation of mutagenic genes and deregulation of signaling pathway frequently lead to the development of cancer. Different subtypes of ovarian cancer reveal different molecular pathways. Coagulation pathway was reported to be disturbed in clear cell ovarian carcinoma (56). Genes that stimulate or inhibit coagulation were noted to be dysregulated. Angiogenesis and glycolysis are two major activated pathways in clear cell ovarian carcinoma (56). Vascular endothelial growth factor (VEGF) and its receptor FLT1 were upregulated in this type of cancer and involved in angiogenesis. Earlier study by Yamaguchi et al 2010, reported molecular pathway related to clear cell ovarian cancer was related to hypoxia-inducible factor 1 (HIF1α) (57). HIF1α regulates ADM, which is related to angiogenesis. It also regulates genes that are linked to glucose metabolism including SLC2A1 in glucose transport and HK1/HK2 and ENO1/ENO2 in glycolysis. Both pathways could act as potential therapeutic target based on the small interfering RNA of genes related to these pathways combined with antiangiogenic drug, Sunitinib(56).

3.2. Gene expression profiling in ovarian cancer

In ovarian cancer study, microarray was used to classify 113 samples from five different histopathological subtypes; endometrioid, serous, mucinous, clear cell and mixed type according to the gene expression pattern (58). The results showed 95% of all samples were clustered within their expected groups. Gene expression profile in this study failed to distinguish between high-grade endometrioid and serous ovarian cancer. The result derived from the principal component analysis demonstrated the separation of celar cell, mucinous and endometrioid with serous ovarian cancer. This can be explained through the origin of these types of cancer, which is Mullerian epithelium. In contrast to serous ovarian cancer, which most likely arise directly from ovarian surface epithelium (58). Microarray was also used to distinguish between various grades of clear cells ovarian cancer from other subtypes of ovarian cancer including serous papillary (59). Among genes identified were E-cadherin and osteonidogen were detected at high level in clear cells. While discoidin domain receptor family member (DDR1), estrogen receptor 1 and cytochrome P450 4B1 were at a low level in clear cells ovarian cancer compared to other ovarian cancers (59).

A separate microarray study was done on 285 of various grades of endometrioid and serous ovarian cancer samples that were analysed together with low-grade serous and endometroid ovarian cancer (60). The result showed high-grade serous subtype was related to overexpression of Wnt/βcatenin and cadherin pathway genes including N-cadherin and P-cadherin but low E-cadherin protein expression. This finding demonstrated the high-grade serous ovarian cancer contained messenchymal expression pattern. Also it has suggested there is epithelium-mesenchymal transition in this subtype of ovarian cancer. High expression of genes related to proliferation and extracellular matrix-related genes such as COL4A5, COL9A1 and CLDN6. Immune cell markers such as CD45, PTPRC and lymphocyte markers, CD2, CD3D and CD8A were expressed low in the high-grade serous subtype (60).