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Frontiers in Anti-Cancer Drug Discovery

Volume 7

Editors:
Atta-ur-Rahman, *FRS*
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Frontiers in Anti-Cancer Drug Discovery

(Volume 7)

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PREFACE

Cancers are major causes of death today. Cancer incidents are far greater now than at any time in human history. Despite vigorous research in the fields of cellular and molecular biology of cancer cells, cancer genetics, cancer related proteomics, transcriptomics, and metabolomics, as well as medicinal chemistry, the successful treatment of various cancers has met with limited success. The main issue has been our inability to translate of most of the basic research discoveries into medicines that can substantially improve the treatment of many cancers. The problems are even more complex in cancer forms which are either very rare or highly heterogeneous. Cancer treatment thus is a grand challenge of our times. Scientists, and health care professionals in the academia, government, and in industries are striving to meet this mega challenge of the 21st century, apparent from the huge number of basic and epidemiological research publications. This has created a need of a focused book series, based on reviews of current literature by leading experts. The *e*Book series *"Frontiers in Anti-Cancer Drug Discovery"* is a humble effort to cater to this need.

The 7th volume of this internationally recognized books series comprises five comprehensive reviews, written by leading practitioners in this field. Each review is focused on certain important aspects of anti-cancer drug discovery and development, including identification of new molecular targets, and outcomes of pre-clinical and clinical studies on new drugs, and combination therapies.

Callejas González *et al.* have reviewed recent pre-clinical and clinical studies on various drugs against malignant pleural mesothelioma (MPM). It is a rare but highly aggressive cancer of the linings of the lungs and the chest wall, known as the Pleural mesothelioma, caused by exposure to asbestos. Apart from surgical removal of the affected part, combination of chemotherapy, such as pemetrexed and cisplatin, is known to increase the median survival rate in MPM patents. The authors have extensively reviewed the results of various pre-clinical and clinical studies conducted on new and known combinations of chemotherapeutic agents against MPM. This includes mesothelin specific antibody and toxin therapies, gene therapy, interleukin-4 receptor toxins, dendritic cell vaccines, *etc*. The emphasis has been on improving the evolution of the disease and development of better clinical models for treatment.

The review contributed by Preto *et al.* gives a comprehensive account of the various therapeutic options available for the treatment of highly malignant and complex colorectal cancer (CRC). Starting from a detailed introduction of the disease, the authors have systematically reviewed the issues related to the conventional chemotherapeutic agents, such as specific EGFR signalling antagonists, including emerging resistance. This is followed by

an interesting commentary of anti-cancer properties of short-chain fatty acids (SCFA), produced by symbiotic bacteria in colorectal environment. The potential of SCFA in the prevention and treatment of CRC is an interesting area of research. The potential of new class of metabolic inhibitors of glycolytic metabolism in colorectal cancer cells has also been discussed as new therapeutic adjuvants, in combination with other therapies. The review ends with a critical analysis of the literature on the development and use of nanoparticles for cancer therapies, for reducing their adverse effects.

Pancreatic cancer has a poor prognosis, even if diagnosed early. It spreads rapidly and is rarely detected in its early phases, making it a leading cause of cancer death. Surgical removal of cancerous pancreas, before metastases, is the most practiced treatment regimen. Chemotherapy against pancreatic cancer, though used frequently, has many limitations. Sridhar *et al.* have reviewed the most recent R&D on emerging new therapies against pancreatic cancers. The new drugs specifically target various growth factors and signalling molecules, including epidermal growth factors, and hedgehog signalling pathway. The pre-clinical and clinical outcomes of these drugs are presented in this article.

The review by Georgios M. Iatrakis is focussed on the role of adjuvant endocrine therapies in early breast cancer (BC) treatment. Endocrine adjuvant therapy plays an important role in the prevention, recurrence and treatment of hormone receptor-positive breast cancer. It has been shown to reduce the risk of recurrence and death from breast cancer. Tamoxifen (Tx) is a selective estrogen receptor modulator. Recent clinical trials on prolonged (10 years) Tx treatment of BC in certain categories of BC patients has led to significant reduction in mortality due to recurrence. Similarly, treatment with aromatase inhibitors (AIs), such as anastrozole, exemestane and letrozole has shown to have clinical outcome, often superior than the Tx. The author discuss in greater length the comparative advantage of the use of Tx *versus* AIs in various types of breast cancers.

Cyclin E is a member of the cyclin family of proteins. It binds to G_1 phase Cdk2, which is required for transition from the G_1 to S phase of the cell cycle that regulates cell division. At the molecular level, liver cancer is characterised by a disruption of cell cycle regulation through many molecular mechanisms, including over-expression of cyclins (cyclins D, A, E, and B). The review contributed by Nikki P. Lee presents the role of cyclin E in the regulation of cell cycle during liver cancer, focusing mainly on hepatocellular carcinoma (HCC). The potential use of cyclin E for HCC prognosis and treatment is also highlighted and discussed.

In the end, we wish to express our gratitude to the authors of the above cited reviews for their excellent scholarly contributions for the 7*th* volume of this eBook series. We also appreciate the efforts of the entire team of the Bentham Science Publishers for efficient processing. The skills and efforts of Ms. Fariya Zulfiqar (Assistant Manager Publications), Mr. Shehzad Naqvi (Senior Manager Publications) and the leadership of Mr. Mahmood Alam (Director

Publications) are greatly appreciated. We also hope that like the previous volumes, the current volume will also receive wide readership and recognition.

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CHAPTER 1

Malignant Pleural Mesothelioma (MPM): Latent Disease

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Abstract: Malignant pleural mesothelioma (MPM) is a cancer with aggressive nature and poor prognosis (the median survival ranges from 9-18 months). The worldwide incidence of this disease is increasing, with 2180 estimated new cases diagnosed in the United States in 2013. Despite the apparent benefits offered by the multimodal approach (a combination of surgery, chemotherapy -cisplatin/ pemetrexed- and radiotherapy), survival remains poor. As a consequence, multiple therapies aiming to improve the evolution of the disease are under investigation. In this chapter, we will summarize some of the new preclinical and early clinical developments in the treatment of MPM, which include mesothelin specific antibody and toxin therapies, gene therapy, interleukin-4 (IL-4) receptor toxins and dendritic cell vaccines, among others.

Keywords: Activation of invasion, Activation of metastasis, Angiogenesis induction, Avoiding immune destruction, Cancer therapy, Chemotherapy, Cryotherapy, Dendritic cells vaccine, Deregulating cellular metabolism, Enabling replicative immortality, Evading growth suppressors, Genome instability, mutations and epigenetic dysregulation, Intraoperative hyperthermic chemotherapy, Iodine-povidone lavage, Malignant pleural mesothelioma (MPM), Photodynamic therapy, Radiotherapy, Resisting cell death, Surgical treatment,

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Sustaining proliferative signaling, Tumor promoting inflammation, Vaccines and immunotherapy.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a term used to refer to aggressive tumors involving mesothelial cells. These cells normally cover body cavities, especially pleura, peritoneum, pericardium and testicles. Over 90% of cases of mesothelioma occur in the pleura, compared to 4-7% appearing in the peritoneum and less than 1% in the pericardium and testicles [1].

MPM is a malignancy with low prevalence, highly variable clinical presentation and often requires an individualized approach. As a result, conclusions must usually be extracted from small series, retrospectives, specific studies and subjective experiences.

Prognosis of MPM is poor due to the difficulty in establishing an early stage diagnosis, as a result of its rapid progression, high capacity of invasion and the lack of effective treatments [1].

Historical Facts and Etiology

MPM is located in the pleura in 90% of cases, the visceral pleura in 80% and the parietal pleura in 20%. Its association with asbestos is well established since the 50s [2]. In approximately 80% of mesothelioma cases, there is a causal link with occupational exposure to asbestos, involving a wide range of occupations [2].

MPM has been considered an occupational illness. Historically, occupations related to this disease have been categorized into three different groups depending on exposure type. Initially, it was mainly diagnosed in mine and mill workers who were directly exposed to asbestos. Secondly, MPM was diagnosed in plumbers, carpenters, personal defense and insulation installers [2]. And lastly, in people living in areas with exposure to atmospheric asbestos fibers located close to asbestos mines or factories.

In addition, a dose-response relationship between cumulative exposure (high levels of exposure, duration of exposure or both) and MPM has been

demonstrated. Likewise, no threshold below which no risk of contracting the disease exists has been identified [1, 3]. Usually, patients with peritoneal mesothelioma have suffered more intense exposure than patients with pleural disease [4, 5].

The first histological description of MPM malignancy was made by *E. Wagner* in 1870. However, MPM was not associated with asbestos until 1960, as a result of the industrial development occurred during *World War II*, which led to a global increase in the use of this mineral. As a result of his work in South Africa –a country extracting the three commercial types of asbestos–, *JC Wagner et al*. described MPM as a disease with a long latency period affecting mainly miners and mill workers, especially when exposed to crocidolite [6].

Musk *et al*. have conducted a study in Wittenoom (Western Australia) with 7000 mineworkers that had been exposed to blue asbestos (crocidolite) between 1943 and 1966 [7]. At the end of 1986, 94 cases of MPM had been identified, while between 1987 and 2015, there are 692 new documented cases. This industrial disaster is considered the worst of its kind in the history of Australia [7].

Asbestos is the common name given to a group of natural silicates which can be found in soil and can be classified into two types:

- Serpentine (chrysotile or “white asbestos”)
- Amphibole (crocidolite “blue asbestos”), tremolite, amosite “brown asbestos”), anthophyllite and actinolite).

The type of asbestos fiber is a critical risk factor involved in the development of MPM. Serpentine fibers are large, flexible and curly, and they tend to stay in the conducting airways of the respiratory tract. In contrast, amphibole fibers tend to be short, straight and rigid, and move into the lymphatic system of the lung parenchyma, accumulating in the interstitial spaces and the subpleural region. Amphibole fibers, especially crocidolite, are clearly related to MPM; while chrysotile has been more related to lung cancer [8, 9].

The association between MPM and erionite is also known. Erionite is a natural contaminant of the soil that can be found in various regions of the world,

CHAPTER 2

Colorectal Cancer Therapeutic Approaches: From Classical Drugs to New Nanoparticles

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Abstract: Colorectal cancer (CRC) is the second most commonly diagnosed cancer and the third leading cause of cancer related death in the world. Epidemiological studies show that CRC incidence and mortality vary substantially across different regions of the world. CRC is a multifactorial disease process, in which may intervene familial and hereditary factors, as well as age, environmental causes, lifestyle-related risk factors, namely diet and inflammatory conditions of the digestive tract. Several genetic alterations have been associated to the process of colon carcinogenesis, namely epidermal growth factor receptor (EGFR) activation, BRAF and KRAS mutations among others.

Many options for CRC treatment are available, including surgery, chemotherapy, and radiation. Herein, we will describe the main classical drugs used in CRC chemotherapy, such as 5-Fluoracil, Leucovorin, Irinotecan, and Oxaliplatin. Recent anti-CRC therapies are now targeting specifically signaling pathways implicated in colorectal carcinogenesis, such as EGFR (Cetuximab, Matuzumab, Erlotinib, Panitumumab), which appears highly overexpressed in most CRC patient cases. However, this approach is limited by resistance conferred by the activation of mutations in EGFR downstream signaling pathways. As a result, an increasing number of specific components of these pathways have been targeted in order to overcome the resistance to conventional EGFR-targeted therapies. Despite the recent advances, conventional chemotherapy remains unable to improve the prognosis of advanced or recurrent CRC.

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In colorectal environment, there is a symbiotic relationship between intestinal cells and bacteria from the diet. Indeed, colonocytes metabolize short-chain fatty acids (SCFA), byproducts of anaerobic bacterial fermentation of dietary fiber. These SCFAs play a significant role in maintaining the normal physiological functions of the colon mucosa, but they also have strong anti-tumorigenic properties, such as reduction of cancer cell proliferation and differentiation and stimulation of apoptosis in CRC cells. Here we discuss the exploitation of anti-apoptotic features of SCFA in the development of new prevention and therapeutic approaches.

Changes in cellular metabolism are a crucial hallmark of cancer and CRCs were shown to present a glycolytic phenotype even in the presence of oxygen, phenomenon commonly designated as the 'Warburg effect'. We will also discuss the use of metabolic inhibitors as new therapeutic adjuvants *per se* or in combination with other therapies.

To reduce off-target associated adverse effects and achieve targeted drug delivery in cancer therapy, nanomedicine is emerging as a promising strategy. The transport of classical drugs by nanoparticles has shown great promise in improving drug distribution and bioavailability, increasing the anticancer molecules concentration at the cancer tissue, providing optimal drug delivery, and minimizing drug toxicity. Additionally, targeting CRC cells may be improved by incorporating ligands for cancer-specific surface receptors such as EGFR, bringing new opportunities in the treatment of patients with CRC.

Keywords: Bevacizumab, Cetuximab, Classical chemotherapy, Colorectal cancer, 5-Fluoracil, Irinotecan, Metabolic targets, Nanoparticles, Oxaliplatin, Panitumumab, Target therapy.

OVERVIEW ON COLORECTAL CANCER

Epidemiology and Risk Factors of Colorectal Cancer

Colorectal cancer (CRC) is one of the most common cancers worldwide, accounting for over 1 million cases and about half a million deaths annually [1, 2]. CRC is the third most frequent cancer in men, after lung and prostate cancers, and it is the second most frequent cancer in women after breast cancer [3].

Epidemiological studies show that CRC incidence and mortality vary substantially across regions of the world [4 - 6]. The highest incidence is in Western Europe,

North America, Australia and New Zealand [6]. In Europe, CRC is the second cause of death among all cancer types in both genders [3], with ~446.000 new cases arising each year [3, 7]. The incidence rate of CRC is low up to the age of 45-50 years [3], but progressively increases with age. Furthermore, the incidence is higher in the Western world, especially among populations that adopt Western-style diets [1, 4, 8, 9].

CRC is a multifactorial disease process, in which may intervene familial and hereditary factors, age, environmental lifestyle-related risk factors (diet, physical inactivity, obesity, smoking and alcohol consumption), as well as inflammatory conditions of the digestive tract [4].

Approximately 20% of all CRC cases can be attributed to heritable gene variations. In this case, some patients are predisposed to develop CRC: patients with hereditary conditions such as familial adenomatous polyposis (FAP), hereditary non-polyposis CRC and ulcerative colitis [3]. However, the largest fraction of CRC cases is sporadic and linked to environmental causes [3, 4, 8, 9]. The most common risk factors of non-hereditary CRC include upper-middle socioeconomic status and dietary regimens rich in proteins, especially those with high intake of red meat, processed meat and animal fats and usually poor in unrefined cereals and fibers [2, 4, 8]. The association between high intake of red and processed meat and CRC risk has been associated with the content of the meat and the compounds generated by the cooking process (e.g. N-nitrous compounds) [8]. These factors can affect the large intestine mucosa with genotoxicity and metabolic alterations that contribute to colon carcinogenesis [6, 8]. As for smoking tobacco it acts on the intestinal mucosa through the production of carcinogenic agents such as acetaldehyde, benzopyrenes, aromatic amines and N-nitrosamines and by increasing the risk of microsatellite instability [4, 6]. Excessive alcohol consumption has been showed to accelerate CRC initiation and progression by inflammation and epigenetics [4].

On the other hand, CRC may be easily prevented with a dietary fiber consumption [10]. Many of the benefits derived from fiber consumption can be attributed to the anaerobic bacterial fermentation of dietary fiber into short chain fatty acids (SCFAs) [10]. The three major colonic SCFAs are butyrate, propionate and

CHAPTER 3

The Latest Developments in Anti-Pancreatic Cancer Drugs: *A Promising Future Ahead*

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Abstract: Pancreatic cancer has been increasingly diagnosed in the recent decades. Although the cornerstone treatment for pancreatic cancer is surgery, unfortunately, only 15-20% has a resectable disease at the time of initial diagnosis. Owing to the majority of patients having either locally advanced diseases or metastases, chemotherapy plays an important role in the management of pancreatic cancer. Because conventional chemotherapy has its limitations in the management of pancreatic cancer, recently developed molecular targeted therapy has emerged as an important modality of treatment of the disease. Drugs targeting growth factors such as epidermal growth factor and anti-angiogenesis have been studied and have promising results. Some of these drugs such as erlotinib have been already approved for the treatment of pancreatic cancer. Newer drugs such as those targeting hedgehog signaling pathway are now being tested. In this chapter, we will comprehensively review the current and also the latest development of anti-pancreatic cancer drugs.

Keywords: Angiogenesis, Chemotherapy, Growth factors, Molecular, Pancreatic cancer.

INTRODUCTION

The incidence of all types of pancreatic cancer, of which pancreatic adenocarcino-

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ma accounts for 85%, is reported from 1-10 cases per 100,000 persons throughout the world and has remained stable over the past three decades when compared Pancreatic cancer is the disease of the to the incidence of other common solid tumors. The incidence is higher among men and in the developed countries [1, 2]. Worldwide, pancreatic cancer is the eighth leading cause of death in men and the ninth leading cause of death in women from cancer. In the United States, it is the fourth leading cause of cancer death with the estimated 36,888 deaths in 2010. In 2014, the estimated new cases of pancreatic cancer in the United States were 46,420 (23,530 in males and 22,890 in females) and estimated deaths were 39,590 (20,170 in males and 19,420 in females) [3]. Pancreatic cancer is the disease of the elderly and rarely in persons younger than 40 years (Fig. **1**).

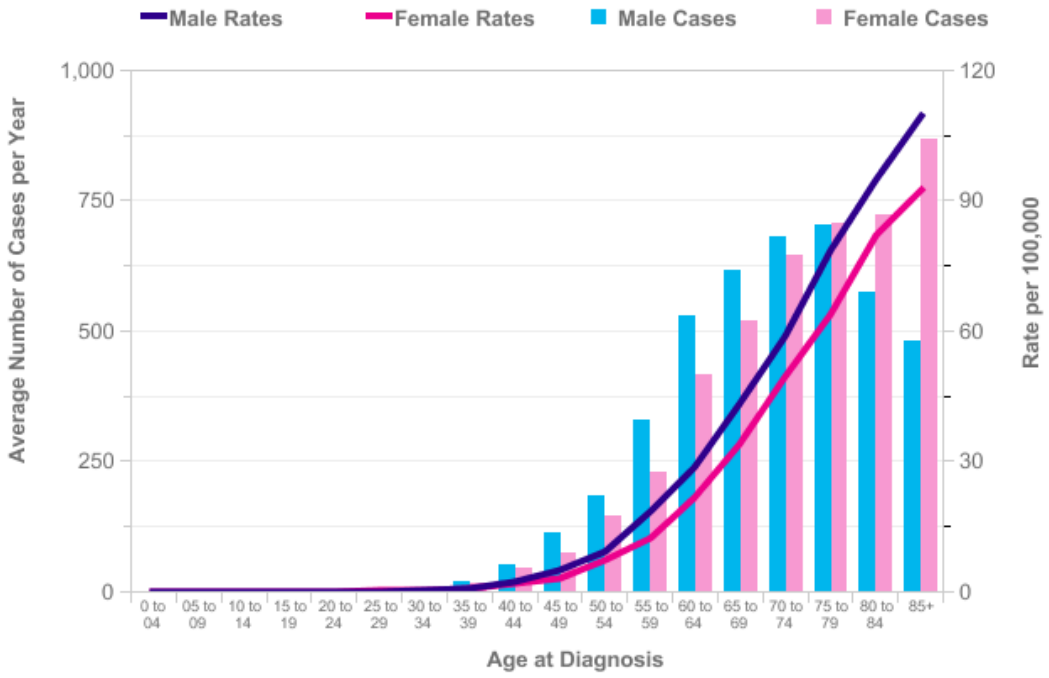

Fig. (1). Incidence of pancreatic cancer per age and gender.

The localized and potentially curable pancreatic cancer is found in less than 20%

of cases [4]. The focus of this review will be on pancreatic adenocarcinoma and the current and also the latest development of anti-pancreatic cancer drugs.

Origin of Pancreatic Cancer Cells

Tumors are largely classified by histological appearance, yet morphologic features do not necessarily predict a lineage relationship [5]. Pancreatic ductal adenocarcinoma (PDAC) has long been considered to arise from pancreatic ducts based on its morphology and the occurrence of dysplasia in putative preneoplastic ductal lesion. Mounting evidence suggests that PDAC and its noninvasive precursor lesion known as pancreatic intraepithelial neoplasia (PanIN) may be the result of acinar cell metaplasia to a ductal cell form [6 - 8]. Genetically engineered mouse models based on tissue-specific *KRAS* activation and deletion of tumor suppressor genes show that PDAC appears to arise from exocrine acinar rather than ductal cells, *via* a process of phenotypic reprogramming that is Sox9-dependent [6]. Targeting of oncogenic *KRAS* mutations to elastase and Mist1-expressing acinar cells of adult mice resulted in the spontaneous induction of PanIN lesions [7]. Similarly, conditional expression of oncogenic *KRAS* along with Notch using *Pdx1* Cre recombinase synergistically caused mature acinar cells to convert to PanIN lesions [8]. These results may imply that therapeutic targeting of signaling pathways involved in ductal reprogramming of acinar cells could prevent PDAC initiation in high-risk individuals.

Molecular Biology of Pancreatic Cancer

Pancreatic cancer is a highly heterogeneous disease with a complex tumor microenvironment [9]. The genetic and cellular heterogeneity within pancreatic tumors may account for its aggressiveness. Histologically, PanIN is a well-defined precursor of pancreatic cancer [10]. The development of minimally dysplastic epithelium (PanIN 1A) to more advanced dysplasia (PanIN 3) and eventually to invasive carcinoma is the result of sequential accumulation of molecular alterations. These molecular changes include: activation of the *KRAS2* oncogene, inactivation of the tumor suppressor gene *CDKN2A* (encodes the inhibitor of cyclin dependent kinase 4 *INK4A*), and, finally, inactivation of the tumor suppressor genes *TP53* and Deleted in Pancreatic Cancer locus 4 (*DPC4*, also

CHAPTER 4

Adjuvant Endocrine Therapy for Early Breast Cancer

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Abstract: Hormone receptor (HR) positive breast cancers (BCs) represent the vast majority of BCs with estrogen receptor (ER)+ and/or progesterone receptor+ BCs comprising the vast majority of all cases. Tamoxifen (Tx) is a selective estrogen receptor modulator that is mainly indicated for premenopausal women with HR+ BCs (as monotherapy), resulting in a significant reduction of BC mortality. The "classical" duration of Tx therapy was 5 years. However, the results of recent trials indicate that 10 years of adjuvant Tx reduces further BC mortality. In premenopausal women, available data suggest that ovarian suppression provides no additional benefits for women treated with adjuvant Tx with the exception of women who are at sufficient risk for recurrence to warrant adjuvant chemotherapy and who remain premenopausal. Aromatase inhibitors (AIs)-anastrozole, exemestane and letrozole-have become a useful adjuvant therapy in the management of postmenopausal patients with HR+ BCs, which proved superior to Tx. Recent guidelines recommend a 5-year course of AIs, administered as initial monotherapy or after Tx, for postmenopausal women with HR+ early BCs. In premenopausal women, it is not appropriate to administer AIs as monotherapy because the low levels of estrogen could stimulate hypothalamos-pituitary axis which could trigger the ovaries for estrogen production. Taking into account the superiority of AIs to Tx, a logical hypothesis was to use AIs in premenopausal women after inducing a menopausal status with GnRH analogues. However, the use of AIs is not generalized in premenopausal women (where Tx is beneficial) but only in high risk cases (women ≤35 years old, and/or large tumor, high tumor grade, involved lymph nodes, lympho-vascular invasion, high recurrence score), where exemestane proved beneficial.

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Keywords: Adjuvant endocrine therapy, Anastrozole, Aromatase inhibitors, Early breast cancer, Exemestane, GnRH analogues, Letrozole, Tamoxifen.

INTRODUCTION

Breast cancer is the main cause of death in women of advanced reproductive age (40 to 49 years) [1]. Hormone receptor (HR)–positive breast cancers represent the vast majority of breast cancers with estrogen receptor (ER) positive and/or progesterone (PR) receptor-positive breast cancers comprising 60-80% of all cases [2, 3]. Actually, molecular subtypes luminal A (ER-positive and/or PR-positive, HER2-negative with low Ki-67) and luminal B (ER-positive and/or PR-positive, HER2-negative with high Ki-67 or HER2-positive) comprise the vast majority of breast cancers with a prevalence of 30-70% and 10-20% respectively. Recently, it was reported that reproductive events in adolescence have differential impact on the risk of breast cancer molecular subtypes [4].

In more detail, about ≤80% of breast cancers are estrogen-receptor positive and about 65% of estrogen-receptor-positive breast cancers are also progesterone-receptor-positive. A small percentage of breast cancers (~2%) are estrogen-receptor-negative and progesterone-receptor-positive. While the clinical utility of ER as a predictive biomarker to identify patients likely to benefit from hormonal therapy is well-established, the added value of PR is less well-defined and controversial [5, 6]. In multivariate models considering ER status and clinico-pathologic factors, routine PR testing was not associated with prognosis, as an independent factor, and its use has been questioned [5]. In fact, estrogen receptor negative/progesterone receptor positive breast cancer was not considered a reproducible subtype. On the contrary, absence of PR expression is considered a powerful, independent prognostic variable in certain cases of operable, primary breast cancer [6]. Probably, different factors can influence the lifetime risk of hormone-receptor-positive or hormone-receptor-negative breast cancer, including diet [7].

Among other systems, Allred scoring is a semi quantitative system that takes into consideration the proportion of ER positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3). The proportion and intensity are

added together to produce total scores of 0 to 8. A (total) score of 0-2 and a score of 3-8 are regarded as negative and positive respectively for ER receptors [8]. In clinical practice, another way to describe HR status is to report the percentage (%) of HR-positive cells and their staining intensity. Taking into account that manual assessment of ER status from breast tissue microscopy images is a subjective, time consuming and error prone process, automatic image analysis methods were proposed [9]. Furthermore, quantitative *in situ* measurement of ER mRNA predicts response to endocrine therapy.

Classification of breast cancer by immunohistochemical expression of estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2) [10] and Ki-67 (protein) is standard practice for clinical decision-making [11].

Ki-67 is an excellent cellular marker for proliferation which determines the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells is often correlated with the clinical course of breast (and other) cancers. In breast cancer, a result of <10% is considered low, 10-20% borderline, and a result of >20% is considered high. Among others, Ki-67 is included in the prognostic factors of overall survival in patients with early hormone receptor-positive invasive ductal breast carcinoma [12]. In addition, high levels of HER2 expression identify those women who benefit from treatment with agents that target HER2. However, HER2 positivity may be associated with resistance to endocrine therapies [10].

It seems that inherited or external factors can influence the percentage of ER-positive tumors. As an example, patients with ≥2 full-term pregnancies exhibit higher percentage of ER-positive tumors, compared with patients who experienced <2 times full-term pregnancies. Similarly, patients whose age of menarche was ≥15 years exhibited a greater chance of PR-positive tumors compared with those whose age of menarche was <15 years old [13].

Several meta-analyses have demonstrated that endocrine therapy consistently improves prognosis (survival outcomes) for women with (non-metastatic) HR-positive breast cancer. Starting a few decades ago [14], the selective estrogen

CHAPTER 5

Cyclin E and its Potential Use for Liver Cancer Prognosis and Therapy

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Abstract: Liver cancer is an aggressive malignancy developed in the liver. Hepatocellular carcinoma (HCC) is the predominant form of liver cancer worldwide. The prognosis of HCC patients remains poor even with the evolving development on technologies for disease diagnosis, prognosis and treatment. New methods to improve the management of HCC patients should be implemented. Cell cycle deregulation is one key mechanism leading to HCC. Cyclin E is a cell cycle molecule regulating G1 to S phase transition of the cell cycle. The prominent cell cycle regulating function of cyclin E has signified its involvement in HCC when its activity is deregulated. This chapter summarizes the current research on cyclin E in HCC and with a special focus on its underlying, well-studied downstream mechanism involving retinoblastoma-E2F transcriptional factor network. Lastly, research on the potential use of cyclin E for HCC prognosis and treatment is also highlighted and discussed.

Keywords: Cell cycle, Cyclin E, Cyclin E1, Cyclin E2, Cyclin-dependent kinase 2, Drug, E2F, Liver cancer, Marker, Overexpression, Prognosis, Retinoblastoma, Therapy, Treatment, Tumorigenesis.

LIMITATIONS OF CURRENT LIVER CANCER MANAGEMENT

Liver cancer is a global liver disease coupled with high mortality. Each year, more than half a million new cases appear and burden heavily on the healthcare system

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(GLOBOCAN 2012). Hepatocellular carcinoma (HCC) is the predominant type of liver cancer, contributing up to more than 80% of the total liver cancer incidences. The management of HCC is clinically demanding especially for the late diagnosed patients who are in advanced disease stage. Late diagnosis is common in HCC due to the asymptomatic nature of the liver tumor. This situation is further compounded by the suboptimal performance of current screening methods including ultrasonography and serum alpha-fetoprotein (AFP) test. For very early and early stage patients, curative resection including tumor resection and liver transplantation remains the frontline treatments. However, these treatments are not suitable for late stage patients and those with metastatic or unresectable tumors. These patients instead are treated alternatively with transarterial chemoembolization, radiofrequency ablation, alcohol injection, molecular targeting therapy, *e.g.* sorafenib and others [1 - 3]. Even with this wide range of treatment options, not all patients can achieve complete cure. Under this clinical situation, it is a long-term goal to research for new strategies to improve the management of HCC in the areas of diagnosis, prognosis and treatment.

Deregulation of Cell Cycle in Tumorigenesis

Cell cycle is a cellular process that generates new daughter cells when a mother cell completes the cell cycle by passing through four phases from G1, S, G2 to M phase. Each phase of the cell cycle represents a preparative stage for the mother cell to acquire essential cellular machinery before the final step of cell division to generate daughter cells. During each cell division, the genetic stability of the daughter cells is tightly maintained and safeguarded by the presence of a distinct set of cell cycle checkpoints. There are four main types of cell cycle checkpoint, composing of G1/S checkpoint, intra-S checkpoint, G2/M checkpoint and spindle assembly checkpoint [4, 5]. In response to any damages or errors occurred during cell cycling, these checkpoints will be activated to halt the progressing cell cycle, such that the damages or errors can be properly repaired before cells entering the next phase of the cell cycle [5, 6]. The presence of this checkpoint mechanism can maintain the genome stability of the dividing cells.

Fine control on cell cycle can sustain physiological processes of cell growth, development and reproduction. Deregulation of cell cycle control, however,

triggers a wide range of diseases in particular cancers. In various types of cancers including HCC, rapid cell cycling coupled with uncontrolled proliferation and multiplication of cancer cells is a hallmark event in tumorigenesis. For HCC, cancer cells lacking control on cell cycle can lead to the formation of tumor nodules in the liver. It is obvious that deregulation of cell cycle in cancer cells belongs to a signature event of liver tumorigenesis. Although it remains unknown whether cancer cell cycle is resulted from failure to control cell cycle or is due to the action of a specific set of cancer-associated cell cycle regulators, a number of studies have reported the involvement of several cell cycle molecules in tumorigenesis. Cell cycle molecules regulating G1 to S phase transition of the cell cycle is frequently found deregulated in tumorigenesis of different types, including lung, breast, head and neck, glioma, pancreas and liver [7]. This observation further implicates the potential use of detecting and controlling cancer cell cycle for diagnosis, prognosis and treatment of HCC.

Cyclin E, A Cell Cycle Molecule Regulating G1 to S Phase Transition

Cyclin E is a key cell cycle molecule regulating G1 to S phase transition of the cell cycle [7, 8] (Fig. **1**). The expression of cyclin E is mainly regulated at the transcriptional level and at the post-translational level [8]. Cyclin E performs its cell cycle-regulating function *via*binding to and activating a protein kinase known as cyclin-dependent kinase 2. Upon binding with cyclin E, cyclin-dependent kinase 2 will phosphorylate a plethora of protein substrates responsible for entry into S phase of the cell cycle. Examples of these substrates are retinoblastoma, nucleophosmin, proliferating cell nuclear antigen and c-Myc. These substrates have immediate roles on cell cycle progression by mediating centrosome duplication, modulating DNA replication and turning on cell cycle-related transcriptional program [8 - 10]. Some of them are indeed tumorigenic molecules themselves, such that their deregulations can also lead to detrimental effects frequently observed during tumorigenesis [10].

Cyclin E has an indispensable role on propagating the cell cycle *via*its effect on regulating the G1 to S phase transition, *i.e.* passing the G1/S checkpoint. Two studies on HCC have confirmed the regulatory role of cyclin E on this aspect. Tsuji *et al.* have revealed higher cyclin E level in cultured HCC cells expressing

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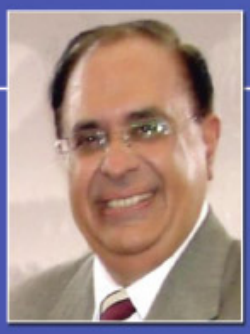

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