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Heide Schatten *Editor*

Cell and Molecular Biology of Ovarian Cancer

Updates, Insights and New Frontiers

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Preface

Ovarian cancer is the second most common gynecological cancer in women, ranging third of the most common cancers among females after breast and lung cancer. Ovarian cancer still has the highest mortality rate being the fifth leading cause of cancer death among women in the United States.

The high mortality rate is in part related to the difficulties to diagnose the disease in early stages, as reliable ovarian tumor biomarkers are still not yet available, and patients do not experience symptoms at early stages of disease development. Late-stage ovarian cancers display various heterogeneous groups of histological features which makes treatment options more difficult and requires more treatment modalities and extensive surgical cytoreduction. Because of the severity of the disease in late stages, it has become an urgent research priority to find new biomarkers and new general health markers to diagnose the disease in early stages and determine effective and specific targeted treatment strategies.

So far, standard treatment for ovarian cancers includes surgery and platinum-based chemotherapy and treatment with taxanes. New clinical trials include the evaluation of new targeted therapeutic agents and immunotherapy combined with primary therapies to increase the survival rate in patients. In addition, extensive screening and research in animal models is aimed at early detection to design new strategies to reduce the currently high mortality rate of ovarian cancer patients and improve the overall 5-year survival rate that remains low at 40% for stage III and 20% for stage IV.

Encouraging new research has been initiated on several levels to combat the disease which in part is based on extensive basic research on cell and molecular biology levels as well as on modification and screening of existing and new drugs to determine more efficient targeted chemotherapies. There are still many unknown factors leading to manifestation of the disease which are currently being addressed in several excellent research labs. New biomarkers are being determined based on the analysis of abnormal signaling events in cancer cells with the goal to repair abnormalities on cellular levels and restore normal cellular functions. It is further being explored why the relapse rate after treatment is still high with relapse occurring in 65–75% of patients. Drug resistance is among the various aspects being investigated to improve currently administered drugs. New approaches also include analyses of general health aspects with new research on the role of the microbiome in disease which is a rapidly evolving highly promising emerging field in the health sciences.

As also indicated in the preface of the companion book on molecular and diagnostic imaging and treatment strategies of ovarian cancer, the advent of molecular and genomic technologies has significantly improved our understanding of the biological processes underlying ovarian cancer which has been enhanced by the Cancer Genome Atlas that has identified mutations in human ovarian cancer genomes that may play a role in tumor progression and modifications of cellular metabolism. Targeted therapies are now available to inhibit specific signaling pathways that are aberrant in ovarian cancer cell populations, and we are now able to image signaling molecules with specific markers in live cells in culture. Progress has also been made in designing nanoparticles that offer the potential for imaging and targeted ovarian cancer treatment. The joint initiatives and efforts of advocate patients, ovarian cancer survivors, basic researchers, statisticians, epidemiologists, and clinicians with various and specific expertise have allowed close communication for more specific and targeted treatment. Major forces supporting these efforts are the Department of Defense, the American Cancer Society, and several other Foundations that recognized the need for intensified advocacy to find treatments for the disease that represents an under-studied area of research. Multi-modal approaches are oftentimes required to manage ovarian cancer and achieve positive outcomes which require patient-specific evaluation and analysis for specific management.

The present book on cell and molecular biology of ovarian cancer is the second one of two companion books, with the second one being focused on specific aspects of cell and molecular biology of ovarian cancer. Both books include new and exciting aspects of ovarian cancer research with chapters written by experts in their respective fields who contributed their unique expertise in specific ovarian cancer research areas and include cell and molecular details that are important for the specific subtopics. Comprehensive and concise reviews are included of key topics in the field. Cutting-edge new information is balanced with background information that is readily understandable for the newcomer, ovarian cancer patients, and for the experienced ovarian cancer researcher alike. Chapters include microtubule-targeting agents: disruption of the cellular cytoskeleton as a backbone of ovarian cancer therapy; tubulin complexity in cancer and metastasis; the impact of centrosome pathologies on ovarian cancer development and progression with a focus on centrosomes as therapeutic target; drug-resistant epithelial ovarian cancer: current and future perspectives; the role of the human microbiome in epithelial ovarian cancer; insights into the microbial composition of intratumoral, reproductive tract, and gut microbiota in ovarian cancer patients; and the impact of mitochondria in ovarian cancer cell metabolism, proliferation, and metastasis.

I hope that this book will stimulate further advances in ovarian cancer research and contribute new insight into potential new targets for ovarian cancer therapies. I am most grateful and would like to express my sincere thanks to the publisher for inviting this book and the companion book on molecular and diagnostic imaging and treatment strategies of ovarian cancer with special thanks to Tiffany Lu and associates for all their excellent help and care during all stages of the project.

It is a great pleasure and timely to edit this book on cell and molecular biology of ovarian cancer depicting areas in ovarian cancer that have impacted new treatment strategies. I am most grateful to the outstanding contributors for sharing their unique and specific expertise with the scientific community. My sincere thanks to all for their most valuable contributions.

Columbia, MO, USA Heide Schatten

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1 Microtubule-Targeting Agents: Disruption of the Cellular Cytoskeleton as a Backbone of Ovarian Cancer Therapy

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Abstract

Microtubules are dynamic polymers composed of α- and β-tubulin heterodimers. Microtubules are universally conserved among eukaryotes and participate in nearly every cellular process, including intracellular trafficking, replication, polarity, cytoskeletal shape, and motility. Due to their fundamental role in mitosis, they represent a classic target of anti-cancer therapy. Microtubule-stabilizing agents currently constitute a component of the most effective regimens for ovarian cancer therapy in both primary and recurrent settings. Unfortunately, the development of resistance continues to present a therapeutic challenge. An understanding of the underlying mechanisms of resistance to microtubule-active agents may facilitate the development of novel and improved approaches to this disease.

Keywords

Microtubule · Tubulin · Dynamic instability · Paclitaxel · Docetaxel · Ixabepilone · Vincristine · Vinblastine · ABC transporter

1.1 Microtubules

Microtubules are cylindrical filaments consisting of α- and β-tubulin subunits which remain universally conserved among eukaryotes and participate in nearly every cellular process, including replication, organelle trafficking, cytoskeletal structure, cell polarity, and motility. Even many prokaryotes have *ftsZ* or similar genes encoding a tubulin homolog that assembles into protein polymers involved in cellular division (Goodson and Jonasson 2018). As such, microtubules are one of the most basic and essential elements of the eukaryotic cell. Mutations resulting in derangement in normal microtubule function contribute to a panoply of human disease, the

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best characterized of which include neurodegenerative disorders such as Alzheimer's disease (Robert and Mathuranath 2007). Given the role of microtubules and their associated proteins in the cell cycle and cytokinesis, it remains unsurprising that dysregulation of microtubule function occurs in many cancers in the setting of constitutive upregulation of proliferation with consequent rapid cell division (Chandrasekaran et al. 2015; Dráber and Dráberová 2021; Hanahan and Weinberg 2011). As a result, antitumor strategies targeting microtubules are used in the treatment of a number of gynecologic cancers, including ovarian, cervical, and endometrial.

1.1.1 Formation and Features

Microtubules are dynamic heterodimeric polymers of α- and β-tubulin. In humans, there are at least nine isoforms of α- and β- tubulin each. Microtubule functional diversity, the "tubulin code," is governed not only by differential expression of the isoforms but also by post-translational modifications (reviewed in the Chap. 2) (Ferreira et al. 2018). Their structure and dynamism are essential to cellular functions, including cytoskeletal organization, mitosis, cell motility, vesicular and organelle transport and signaling, among others. The αβ-tubulin heterodimer pairs are organized head-to-tail (i.e., such that the "head" of a β-tubulin subunit always interfaces with the α-tubulin "tail" of the adjacent αβ-tubulin heterodimer) into 13 protofilaments, which form a hollow polar tube with all heterodimers pointing in the same direction. Lateral interactions between staggered neighboring protofilaments that result in homotypic α-α and β-β contacts provide internal stability of the tube, producing a helical lattice 25 nm in diameter. This internal stability is critical for the dynamic activity characteristic of microtubules because it means that polymerization and depolymerization can only occur at the ends of the tube. The tubes have a plus end, characterized by exposed β-tubulin, and a minus end, characterized by exposed α-tubulin. Heterodimers are added most rapidly to the plus end (Conde and Cáceres 2009; Akhmanova and Steinmetz 2015; Brouhard and Rice 2018). This polarity gives rise to two distinctive microtubule behaviors: dynamic instability and treadmilling. Interestingly, both occur at significantly slower rates in vitro compared to in vivo, highlighting the essential role of microtubule-associated proteins (MAPs) in directing and optimizing the processes (Rodionov et al. 1999; Vasquez et al. 1994).

Dynamic instability Dynamic instability is a stochastic process by which the ends of microtubules rapidly shift between states of growth and shrinkage. Dynamic instability is a form of non-equilibrium polymerization that is facilitated by the hydrolysis of guanosine triphosphate (GTP). Each α- and β-tubulin subunit has a GTP binding site, though only the β subunits hydrolyze GTP. The β-tubulin subunit site to which GTP binds is the E (exchangeable) site. By contrast, the α-subunit binds GTP at the N (non-exchangeable) site where it serves a structural role but is not hydrolyzed (Desai and Mitchison 1997; de Forges et al. 2012; Zhang et al. 2015). When αβ-tubulin heterodimers bind to the plus end of a microtubule, the β-tubulin subunit hydrolyzes GTP to guanosine diphosphate (GDP). As the plus end of the microtubule grows, there will be a considerable portion of the microtubule in which the β-tubulin subunits are bound to GDP. β-tubulin subunits bound to GDP are more prone to depolymerization because of conformational changes to the α-tubulin following GTP hydrolysis of the β-tubulin subunit that place strain on the lattice. If the number of αβ-tubulin heterodimers exceeds the critical concentration (C_c, the amount of monomer necessary to achieve polymerization), a new subunit will be added to the plus end before the previously added subunit is able to hydrolyze GTP. This results in a persistent state of GTP-bound β-tubulin at the plus end. This state, in which an abundance of free subunits permits continual addition to the plus end, is referred to as the *GTP cap* (Cassimeris and Spittle 2001; Brouhard and Rice 2018). The GTP cap thus prevents depolymerization. When the rate of hydrolysis equals or surpasses the rate at which new subunits are added, the result is rapid depolymer-

ization of the microtubule, a process called *catastrophe*. Microscopically, protofilaments of microtubules undergoing catastrophe appear to peel away from the lattice, a behavior that is unsurprising given that microtubules are often observed to have curvature in vivo. Catastrophe is reversed by the process of *rescue*, in which the GTP cap is regained by the addition of new subunits (Desai and Mitchison 1997; Goodson and Jonasson 2018; Brouhard and Rice 2018).

Treadmilling Microtubule growth occurs at both the plus and minus ends, though it does so faster at the plus end. The plus end of the microtubule is more prone to catastrophe than the minus end, while the minus end has a higher rate of rescue than the plus end. Consequently, the predominant activity of microtubules (i.e., growth vs. catastrophe) occurs at the plus end. Treadmilling occurs when the subunits are added to the plus end and lost from the minus end at the same rate. Treadmilling thus represents a steady state for a given microtubule. Microtubules may display primarily dynamic instability, treadmilling behavior, or a mixture of the two (Margolis and Wilson 1981; Rodionov and Borisy 1997; Gadde and Heald 2004; Ganem and Compton 2006).

Treadmilling is a facet of protein polymer behaviors that was first described in actin filaments by Wegner (1976). Microtubule treadmilling has subsequently been demonstrated both in vitro and in vivo (Margolis and Wilson 1981; Chen and Zhang 2004). Additional work has demonstrated that treadmilling is a distinct behavior from dynamic instability and that the two are, collectively, aspects of microtubule dynamics that are essential to broaden cellular functionality of microtubules. Treadmilling of kinetochore microtubules is more specifically called "fluxing" or "poleward flux." This process involves the continual translocation of spindle microtubules poleward coupled to disassembly at the minus ends. Loss of tubulin from the minus ends during metaphase is balanced by treadmilling of units to the plus ends, bound to the kinetochore. The net effect is to maintain constant spindle length while tubulin subunits are constantly flowing poleward (Ganem and Compton 2006; Buster et al. 2007).

1.1.2 Role in Cellular Function

Microtubules, either directly or indirectly, participate in almost every cellular function, including the cell cycle, cytokinesis, intracellular organization, trafficking of organelles, as well as cellular motility and polarity. Their dynamic nature allows them to adapt in response to the changing needs of the cellular environment. Microtubule-associated proteins (MAPs) further influence and regulate microtubule functionality. Mutations affecting microtubules and their functions occur often at the level of MAPs (reviewed in Chap. 2).

Mitosis and Chromosome Segregation One of the most recognized functions of microtubules is in organization of the mitotic spindle (McIntosh 2016). Numerous changes in microtubules must occur as the cell progresses from a resting state (i.e., interphase) to active replication (i.e., mitosis). Interphase microtubules begin to disappear as protein synthesis slows and are replaced by microtubules nucleating from centrosomes. These microtubules are more labile due to phosphorylation of MAPs that regulate dynamics, including stathmin. As the interphase microtubules disappear, the cell becomes more rotund, granting it a symmetry that will be useful later in cytokinesis. During this transition, the centrosome duplicates to produce a bipolar mitotic spindle. As the new microtubules grow, the mitotic spindle forms. Three species of microtubules comprise the mitotic spindle: kinetochore microtubules, astral microtubules, and non-kinetochore (or interpolar) microtubules. Kinetochore microtubules nucleate from the centrosome and the plus ends bind the kinetochore attached to each sister chromatid. The kinetochore is a large, disk-shaped multiprotein structure that attaches to an area of condensed satellite DNA on the chromatid called the centromere. Each kinetochore binds multiple cross-linked microtubules called K-fibers that serve as power-

ful anchors during separation of the daughter cells (anaphase). Astral microtubules radiate from the centrosome but do not bind the kinetochore and serve to help position the mitotic spindle within the cell itself. Non-kinetochore microtubules are densely packed between the poles and crosslink with associated proteins to perform a structural scaffold for the spindle. Motor proteins belonging to the family of kinesins and dyneins bind astral and non-kinetochore microtubules and generate polarity. Kinesins engage in the mitotic spindle. For instance, the tetrameric protein kinesin-5 contains two dimeric motor domains at each end that can be oriented in opposite directions to bind antiparallel microtubules at their respective plus ends and slide them in opposite directions. The result of this is to push the minus ends of the microtubules polewards. Dynein serves two functions in the mitotic spindle: (1) attaching to the minus end of a microtubule formed in the body of the spindle and transporting it to the minus end of one nucleated at the centrosome, resulting in elongation of microtubules nucleated at the centrosome and (2) binding the plus end of astral microtubules and pulling them to the cell cortex, maintaining spindle polarity (Goulet and Moores 2013; McIntosh 2016; Alberts et al. 2017). Following spindle attachment, the aligned chromosomes oscillate under tension due to growth and shortening of the kinetochore microtubules (Shelby et al. 1996). This is coupled with microtubule treadmilling or flux behavior from the poles to the kinetochore during anaphase, which results in the poleward migration of sister chromatids (Mitchison 1989; Chen and Zhang 2004; Buster et al. 2007).

Intracellular Organization The centrosome serves as the microtubule-organizing center (MTOC) of an animal cell. Of note, centrosomes are absent in many fungi and seed plants, many classes of protists, and even mammalian female oocytes (Carvalho-Santos et al. 2011; Courtois et al. 2012). Centrosomes consist of two centrioles, pericentriolar material, and γ-tubulin ring complexes. Centrioles are comprised of nine triplets of specialized microtubules that recruit pericentriolar material. The two centrioles are organized perpendicularly. Pericentriolar material is a matrix that provides structure to the centrosome and contains proteins essential for microtubule nucleation, including γ-tubulin and the accessory proteins that make up the γ-tubulin ring complex (γ-TuRC). The centrosome displays enrichment of γ-tubulin, a specialized tubulin complexed to accessory proteins that helps nucleate microtubules from the MTOC. A centrosome can recruit around 50 copies of γ-TuRC. γ-TuRCs serve as points of nucleation for any microtubule originating from the MTOC; however, because the majority of microtubules are not attached to the MTOC, the cytoplasm contains most of the γ-TuRC in a given cell. Following nucleation, the minus end of the microtubule remains at the centrosome while the plus end grows toward the cell periphery (Akhmanova and Steinmetz 2015; Gadde and Heald 2004; de Forges et al. 2012; Goodson and Jonasson 2018; Kollman et al. 2011). This gives microtubules both an intrinsic negative-to-positive polarity and also a central-to-distal cellular spatial polarity in which the minus ends are located centrally while the plus ends are at the cell periphery. This subsequently confers directionality for intracellular trafficking. This is important because, as discussed below, trafficking in either direction requires motor proteins specific to that polarity.

Organelle trafficking: kinesin/dynein motors Microtubules have two associated motor protein families, kinesin and dynein, that assist with intracellular transport of organelles in opposing directions. Microtubules serve as dynamic tracks along which these motor proteins traffic cargo. Kinesins were the first motor proteins discovered that could move cargo along microtubules (Vale et al. 1985). Since then, the kinesin family has been expanded to include at least 45 distinct kinesin-related proteins in humans. Kinesins share significant sequence homology in their motor domains; however, their unifying function is not transportation of cargo, but rather regulation of microtubule dynamics. Dimeric, hetero-trimeric, and tetrameric kinesins have been characterized. Variation of the kinesin