David A. Frank *Editor*

Signaling Pathways in Cancer Pathogenesis and Therapy

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ISBN 978-1-4614-1215-1 e-ISBN 978-1-4614-1216-8
DOI 10.1007/978-1-4614-1216-8
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011939409

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Chapter 1
Signaling Pathways in Cancer: Twenty-First Century Approaches to Cancer Therapy

David A. Frank

Although descriptions of the disease we now call cancer have been found in ancient writings, useful treatments for malignancies have only been available since the 1940s. The work of Goodman and Gilman at Yale on alkylating agents, and of Sidney Farber in Boston on antifolates, allowed for the first time the reliable regression of advanced cancers, largely leukemias and lymphomas. It soon became apparent that single anticancer agents would generally only lead to transient responses, and as the tumors recurred, they were resistant to repeated treatments with the same agents. Thus, the era of combination chemotherapy arrived, with carefully designed clinical trials, often spearheaded at the National Cancer Institute, testing the effects of various combinations of chemotherapy agents. It was soon apparent that by using complementary mechanisms of action, and avoiding the emergence of resistance, multiagent chemotherapy was considerably more effective than single agents, and previously lethal leukemias and lymphomas could be cured.

In subsequent decades, drugs that targeted other cellular components, such as microtubules and topoisomerases, were added to the armamentarium. In the 1970s, diseases that had been rapidly fatal, like advanced testicular cancer, were now eminently curable. Advances in supportive care, including transfusion of blood products, antibiotic support, and antinausea drug furthered our ability to treat patients with cancer. However, in the 1990s, at the time of celebrations of the 50th anniversary of some of the seminal moments in the discovery of anticancer agents, we had reached somewhat of a plateau. Relatively few new anticancer agents were emerging, and those that were being approved were often just analogues of prior agents. For some cancers, like acute myelogenous leukemia (AML), the most lethal form of leukemia in adults, we were still using the same two cytotoxic agents we had

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D.A. Frank (ed.), *Signaling Pathways in Cancer Pathogenesis and Therapy*,
DOI 10.1007/978-1-4614-1216-8_1, © Springer Science+Business Media, LLC 2012

been using for decades. Advanced forms of common cancers, such as cancers of the lung, breast, prostate, colon, and pancreas remained incurable, and approximately 500,000 people were dying of cancer per year in the United States.

However, as the twentieth century was ending and the twenty-first century was beginning, a very different approach to cancer therapy was being reported in both scientific journals and local newspapers. A new treatment had emerged for a relatively rare blood cancer, chronic myelogenous leukemia (CML). Decades of research had shown that nearly every patient with CML had a translocation between chromosomes 9 and 22, leading to the fusion of two genes, *Bcr* and *Abl*, leading to the production of a chimeric protein, Bcr/Abl. This was a highly active tyrosine kinase that phosphorylated a range of cellular substrates, and drove the malignant behavior of the leukemic cell. Through a combination of solid scientific work, clinical acumen, and personal drive, Brian Druker and colleagues developed a drug, imatinib mesylate, often referred to by its trade name, Gleevec. Imatinib, a pill taken once daily, inhibited the tyrosine kinase activity of Bcr/Abl, and rapidly reversed the signs and symptoms of leukemia in the great majority of CML patients who took it. Although "miracle cures" seem to occur only in movies, for many patients with CML, imatinib was truly miraculous.

The success of imatinib raised great hope that other cancers would be vanquished in a similar fashion. In some rare cancers, like gastrointestinal stromal tumor (GIST), an activating mutation in another kinase, c-kit, was found, and patients with these mutations often had a dramatic response to imatinib and other tyrosine kinase inhibitors. Subtypes of common cancers were also found to have mutations that could be exploited therapeutically, like Her2 amplification in breast cancer (which can be treated by both drugs that block its activity and antibody-based therapies) or mutation of the epidermal growth factor receptor (EGFR) in non-small cell lung cancer (which can be treated with drugs blocking its inappropriately activated kinase).

These triumphs represented the fruits of years of basic research focused on uncovering the molecular underpinnings of cancer. However, we still have 500,000 Americans dying each year of cancer, and the challenge now is to extend this paradigm of basic discovery being translated into effective therapies. It is with that background in mind that this volume is particularly timely. The goal was to recruit experts on many of the key pathways whose function or diversion plays a critical role in the biology of a cancer cell, with a particular thought as to how one can then use their knowledge to consider therapeutic applications that can be offered to patients. Recognizing that every scientific sector has much to contribute in this area, a multinational team of authors, working in industry, government, and academia was asked to highlight key areas for a twenty-first century approach to cancer therapy, based on an intimate knowledge of the workings and derangements of a cancer cell.

Each of the chapters in some ways weaves together basic biology and early approaches to cancer therapy with the most current and sophisticated approaches being developed. Starting with a focus on antimitotic agents, we start with a consideration of tubulin-targeting agents, such as vinca alkaloids, which represent some of the first anticancer agents given to patients, and end with drugs targeting specific kinases and other enzymes that regulate key steps in mitosis. This is followed by a review of the signaling events surrounding DNA damage which provides insight into both the pathogenesis of cancer, and unique ways in which cancer cells could be targeted.

The next chapter also takes a historical perspective, starting with observations made by Peyton Rous on animal tumors in the early 1900s to our current understanding of the role that Src and its related tyrosine kinases play in normal cellular function and tumor pathogenesis, and as targets for current cancer therapy.

Reflecting on the importance of basic biologic research, including developmental studies in "lower" organisms, we now understand that pathways named for phenotypes in Drosophila, such as Wingless and Hedgehog, are important in tissue homeostasis in mammals, and in the development of cancer in humans. Once again, this knowledge opens up a number of opportunities for targeted rational therapy for patients, which has the potential to be both more effective and less toxic.

While identification of the mutations occurring in a cancer cell will hopefully lead to therapies directly targeting these molecular events, such as imatinib for CML, most common human cancers have a large number of mutations, and it can be difficult to deconvolute which are of critical importance, and exactly how they drive malignant cellular behavior. However, these mutations often lead to the activation of signaling pathways which converge on a relatively small number of transcription factors, such as STATs. While STATs themselves are not mutated in cancer, by integrating signals from multiple pathways, they represent excellent targets for cancer therapy.

Finally, as biological research uncovers targets that might be particularly useful in treating cancer, the key question arises as to how can one take this knowledge and actually develop a therapeutic agent that can be given to a patient. The final chapter was written by Michael Corbley, a uniquely talented scientist who has comprehensively reviewed the broad topic of protein therapeutics for cancer, an exciting and dynamic area of therapeutic research. Amazingly, Michael wrote this chapter while he himself was battling advanced cancer. Tragically, Michael died shortly after completing this work. In some ways, Michael's courage, strength, and commitment encapsulates where we are with cancer therapy in the second decade of the twenty-first century. We have wonderfully talented and dedicated researchers who are putting their enormous talents to work at the interface of scientific discovery and clinical medicine. At the same time, we have incredibly strong and brave patients who very much need more effective, less toxic, rationally designed cancer therapies. Through both Michael's wisdom shared in these pages and the inspiration of his own battle with this disease, it is hoped that this volume will provide another step upward toward our shared goal of making cancer an eminently controllable disease, and thus it is to Michael Corbley that this book is dedicated.

Chapter 2
Current and Next Generation Antimitotic Therapies in Cancer

Jeffrey A. Ecsedy, Mark Manfredi, Arijit Chakravarty, and Natalie D'Amore

2.1 Current Therapeutic Application of Antimicrotubule Agents

The neatly ordered, symmetrical appearance of the microtubule spindle during mitotic cell division belies the highly dynamic nature of this critical event during mitosis. In organizing the mitotic spindle and executing a successful division, a wide array of proteins cooperate to line up and then move chromosomes along their microtubule scaffolds (Fig. 2.1). The disruption of the mitotic machinery as a chemotherapeutic approach therefore has the potential to cause cancer cell death or arrest without affecting normal, nondividing tissue. Traditional antimitotic agents comprise those that directly interfere with microtubule dynamics, essential for mitotic spindle assembly and the subsequent alignment and segregation of DNA to daughter cells. Antimicrotubule agents currently being used in clinical setting are the taxanes, vinca alkaloids, and epothilones. These agents are used in a host of cancer types as single agents and in combination with other oncology therapeutics.

Paclitaxel (brand name Taxol), the first taxane identified, was discovered in extracts of bark from the Pacific yew tree in the early 1960s and was approved for the treatment of ovarian cancer three decades later in 1992. Docetaxel (brand name Taxotere) is a semisynthetic derivative of paclitaxel that is more soluble and has demonstrated distinct clinical activity in some cancers, including metastatic breast cancer (Jones et al. 2005). In general, paclitaxel and docetaxel have a similar spectrum of clinical activity including ovarian, lung, breast, bladder, and prostate cancers. Even though both paclitaxel and docetaxel have been used clinically for many years, their utility continues to expand into new indications and in new combinations with other agents.

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D.A. Frank (ed.), *Signaling Pathways in Cancer Pathogenesis and Therapy*,
DOI 10.1007/978-1-4614-1216-8_2,

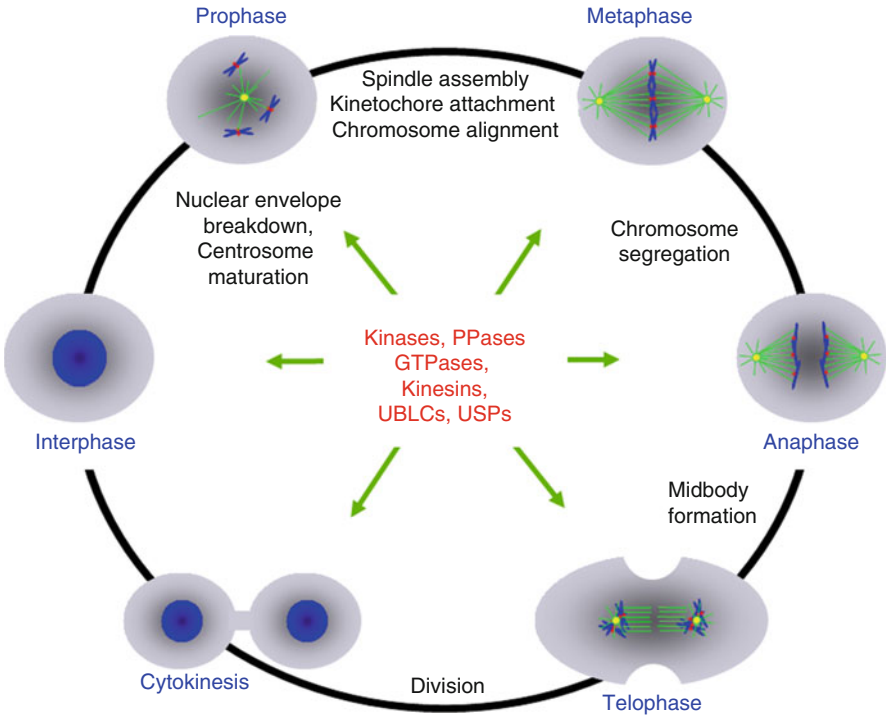

Fig. 2.1 Overview of normal progression through mitosis. A diverse array of kinases, phophatases (PPases), GTPases, kinesins, ubiquitin-like conjugators (UBLCs), and ubiquitin specific proteases (USPs) orchestrate the various stages of mitosis; including prophase, metaphase, anaphase, telophase, and cytokinesis. Some of the critical events that occur during each of these stages are highlighted

Abraxane™ is paclitaxel formulated in albumin-bound nanoparticles, eliminating the need for Cremephor-EL in the formulation, a vehicle that on its own has demonstrated toxicities and necessitates premedication (Ibrahim et al. 2002). Abraxane was approved on clinical data that demonstrated greater activity and safety than paclitaxel in patients with metastatic breast cancer.

The toxicities associated with each of the taxanes are similar, and include neutropenia as the major dose limiting toxicity, along with significant peripheral neuropathy. In fact, dose reductions are frequent in heavily pretreated patients to mitigate the severity of these toxicities. Interestingly, in clinical studies dose reductions did not reduce the clinical response of the agents, suggesting that the optimal biological dose may be lower than the maximum tolerated dose (Salminen et al. 1999). Weekly administration of the taxanes has become more frequently used as clinical data demonstrated less myelosuppression with no decrease in clinical response (Gonzalez-Angulo and Hortobagyi 2008). Interestingly, in breast cancer studies, weekly paclitaxel showed better response rates than once every 3 week dosing (Seidman et al. 2008). However, weekly paclitaxel has demonstrated greater neuropathy than the every 3 week schedule.

The vinca alkaloids where discovered in the 1950s from extracts of the leaves of the periwinkle plant (Catharanthus roseus). The vinka alkaloids were originally considered for use as antidiabetic agents, however, it was quickly learned that they possessed antiproliferative activity. Vincristine and Vinblastine, both microtubule destabilizers are the oldest and most studied members within this class of microtubule binding agents, and are now standard of care agents in various cancer types. Vincristine is used for treating several tumor types, including Non-Hodgkin and Hodgkin lymphoma and certain pediatric cancers, while vinblastine is used for treating testicular, Hodgkin lymphoma, lung, head, and neck, and breast cancer. More recently vinorelbine, a semisynthetic vinca alkaloid, was discovered to have a better preclinical profile than other family members (Krikorian and Breillout 1991). Vinorelbine was approved for treating NSCLC and has shown promising activity in breast, head and neck, ovarian, and squamous cell carcinoma (Burstein et al. 2003; Jahanzeb et al. 2002). Toxicities associated with the various vinka alkaloid members are similar, with neutropenia and peripheral neuropathy being dose limiting.

The epothilones are a newer class of tubulin binding agents that were first isolated in the 1990s from the myxobacterium *Sorangium cellulosum* (Bollag et al. 1995). There are several naturally occurring (epothilone A, B, C, and D) and semisynthetic variants currently under clinical investigation, with Ixabepilone, a derivative of epothilone B, now approved for the treatment of advanced breast cancer (Fumoleau et al. 2007). Similar to the taxanes, the epothilones promote microtubule stability, and in fact share the same binding site with paclitaxel. The perceived advantages over the taxanes include greater potency and decreased likelihood for resistance resulting from drug pumps and tubulin mutations (Kowalski et al. 1997; Wartmann and Altmann 2002). Moreover, the epothilones are formulated in vehicles that are better tolerated than the cremophor used for paclitaxel (Sessa et al. 2007; Watkins et al. 2005).

There are several differences in the toxicities and clinical activity between the various epothilones. Patupilone is the natural product epothilone B and is in phase III studies versus doxorubicin in ovarian, fallopian tube, and peritoneal cancers. Patupilone demonstrated Phase II single agent activity in several tumor types including colorectal, gastric, hepatocellular, non-small cell lung cancer, ovarian, and renal cancer (Harrison et al. 2009). Unlike the taxanes and other epothilones, diarrhea rather than neutropenia was the major dose limiting toxicity in all the schedules tested (Rubin et al. 2005). Interestingly, there was little neutropenia or significant peripheral neuropathy seen in the trials.

Ixabepilone is a derivative of epothilone B which has greater metabolic stability than the parent natural product. Ixabepilone was approved from a phase II study as a single agent for patients with advanced breast cancer who are resistant to prior treatment with an anthracycline, taxane, and capecitabine (Perez et al. 2007). Ixabepilone has demonstrated activity in bladder, breast, non-Hodgkin lymphoma, non-small cell lung cancer, pancreatic, prostate, renal, and sarcoma (summarized in (Harrison et al. 2009)). Unlike patupilone, in a phase II study ixabepilone failed to demonstrate activity in colorectal cancer suggesting that these agents may have a different spectrum of clinical activity. Ixabepilone completed a pivotal phase III trial in advanced breast cancer in combination with capecitabine where it demonstrated

greater activity than capecitabine alone (Thomas et al. 2007). Particularly interesting was the improved progression free survival in the combination group in patients with triple negative breast cancer, a patient population that has a high unmet medical need. The dose limiting toxicities in the majority of the trials were neutropenia and fatigue. The epothilones represent a promising new class of tubulin-binding antimitotics that have already differentiated themselves from the taxanes.

2.2 Antimitotic Agents: Mechanism of Action

Inhibition of the mitotic machinery results in a diverse array of outcomes, primarily leading to cell death or arrest (Fig. 2.2). As the effect of antimitotic agents is not limited to cancer cells alone, the dose-limiting toxicities of these drugs in a clinical setting frequently manifest in rapidly dividing tissue and are often accompanied by severe peripheral neuropathy in the case of antimicrotubule agents. Therefore, the narrow therapeutic index of antimitotic agents necessitates a precise understanding of the mechanism of action of these drugs to maximize the chances of rational development of these therapies.

Our understanding of the basic science underlying antimitotic therapies has been primarily developed using taxanes, including paclitaxel and docetaxel. Taxanes stabilize microtubules by altering the kinetics of microtubule depolymerization. In mammalian cells grown in culture, high concentrations of paclitaxel cause the aggregation of microtubules (Schiff and Horwitz 1980). At lower concentrations that resemble exposures achieved in clinical settings, the primary effect of paclitaxel is to stabilize microtubules, and thereby dampen the dynamic instability of microtubules that is a requisite for efficient spindle assembly. As a result of this dampening, microtubules are unable to grow and shrink rapidly, and their ability to bind to condensed chromosomes during mitosis is compromised. Efficient chromosome alignment is thus affected, and this failure of chromosome alignment leads to mitotic delays mediated via the spindle assembly

Fig. 2.2 (continued) and their inhibition can lead to delayed mitotic entry. Once in mitosis, perturbation of a variety of targets leads to dramatic abnormalities in centrosome maturation/separation, mitotic spindle formation, chromosome condensation, attachment of microtubules to kinetochores, and spindle assembly checkpoint signaling among other events, leading to chromosome alignment defects. The fate of these cells is varied, and can include apoptosis directly from mitosis, anaphase initiation accompanied by chromosome segregation defects leading to an aneuploid division, or exit from mitosis without cytokinesis via mitotic slippage leading to G1 tetraploid cells (double the normal DNA content at this stage). The interphase cells derived from these abnormal mitotic divisions often present as micronucleated or multinucleated. G1 tetraploid cells may undergo additional rounds of DNA replication via a process referred to as endoreduplication resulting in polyploid cells. Ultimately, these cells will eventually die via apoptosis or become senescent, which themselves can eventually undergo apoptosis. Lastly, if cells survive the events associated with an abnormal division, they can undergo additional rounds of mitotic division