CONCLUSIONS

Microtubules are extremely important entities in the cell. In my thesis I have exposed several of the lesser studied functions of microtubules and have demonstrated the infinite value they possess as a chemotherapeutic target. Microtubule targeting agents are among the most useful agents in the clinic nowadays to combat the dreadful disease of cancer. The standard story that microtubule-targeting agents kill cancer cells by arresting them in mitosis and leading them to apoptosis is only the top of the iceberg as to the full mechanism of microtubule-targeting agents. In my first objective of this thesis I presented the fact that microtubules act as highways throughout the cell that enable proteins to traffic from one area of the cell to another. I focused on two important proteins: p53 and HIF-1α. I showed that upon treatment with microtubule interacting agents a cellular reorganization of the two proteins occurred. In the case of p53 and HIF-1α, the high doses of drug destroyed the microtubule network forcing the proteins to remain in the cytoplasmic area of the cell. Upon damage to the cell, both of these proteins are rapidly translocated to the nuclear compartment to carry out their functions. On the other hand, in the case of p53 we found that very low doses of microtubule-interacting drugs that did not affect the microtubule integrity actually increased the level of p53 in the nucleus by enhancing the nuclear-driven transport along the microtubule network. In the case of HIF-1α we found that not only does the protein travel along the microtubules, but so does the mRNA en route from the nucleus to its site of translation. Therefore the therapeutic mechanism of microtubule-interacting drugs, such as 2Me-2 is two fold: they disrupt the nuclear translocation of nuclear-driven proteins and they disrupt the translation of the protein.

Clinical drug resistance is a major barrier to overcome before chemotherapy can become curative for most cancer patients. In the majority of cases, drug resistance eventually develops and is universally fatal. If this could be prevented or at least overcome, the impact for cancer therapy would be substantial. Rational attempts to tackle clinical drug resistance need to be based on our understanding of the molecular mechanisms involved. These mechanisms are likely to be complex and multifactorial, given the high level of genomic instability and mutations seen in cancer cells, as well as the fact that a combination of chemotherapy agents, each one having a different cellular target, are generally administered clinically. All of these factors together with the high degree of

tumor heterogeneity seen in patients, allow the cancer cell many escape routes to survival. We have described a mechanism by which taxol-resistant cells escape the toxic effects of taxol. In addition to harboring a β-tubulin mutation that inhibits the drug binding to tubulin, they also present a compromised mitotic response to taxol. These cells do die from taxol treatment at high enough doses; however, they do not arrest in mitosis; suggesting an alternate target for the drug within the cell. We found that upon exogenous introduction of survivin, the PTX10 cells responded better to taxol by arresting in mitosis and subsequently dying of apoptosis.

Molecular insights into mechanisms of drug resistance are often achieved through studies of experimental models of induced drug resistance. In the case of taxanes and other microtubule-targeting agents these models primarily include cancer cell lines with induced-resistance phenotypes following drug selection. Although, the role of acquired tubulin mutations in clinical drug resistance is not yet clear, acquisition of tubulin mutations in cultured cells is one of the most frequently occurring mechanisms of resistance to microtubule-targeting agents. Molecular and structural studies of these acquired tubulin mutations have deepened significantly our understanding of the basic biology of tubulin and microtubules and have revealed important information on drug-tubulin interactions. We have established a temporal model for the development of drug resistance in cells selected with taxol and epothilones; however, we speculate that it can be applied to most microtubule-interacting agents, if not all. We hypothesize that a mutation in one allele of beta- tubulin is a first step in the development of drug resistance, followed by loss of heterozygosity in the remaining wild-type beta-tubulin allele, leading to increased levels of drugs resistance. The knowledge obtained from understanding the basic biology of tubulin has provided us with attractive alternative treatments to overcome resistance by using structurally-related compounds whose activity is not affected by the presence of specific tubulin mutations, as is the case of Laulimalide. We found that this microtubule-polymerizing drug binds tubulin at an unknown location. It is an active drug in all resistant cells selected with anti-microtubule drugs tested so far and it evens retains activity in cells with P-gp overexpression. Furthermore, we have investigated the combination of farnesyltransferase inhibitors and taxol, proving and extensively explaining the basis for their effectiveness in taxol-resistant cells and

patients. The treatment of FTIs along with the taxol increases the binding of taxol to the cells, even in taxol-resistant cells, via HDAC6, a tubulin deacetylase.

As microtubule-targeting drugs will continue to play a major role in anticancer therapy, and resistance to these drugs will continue to be an important clinical issue until minimized, we hope that the knowledge gained from these preclinical studies will lead to more effective cancer therapies. Room for optimism is found in current treatments of AIDS, another disease plagued by drug resistance due to mutations in two important clinical targets; namely reverse transcriptase and HIV protease. Drug cocktails targeting different viral proteins, appear often to be effective at causing significant and long-term reduction in viral load (303, 304). A similar strategy has been less successful in the case of bacterial infection. Within this regime, mutations of a variety of bacterial proteins is sufficiently rapid that mono-drug therapies are consistently playing catch-up (303, 304). In some instances, control of clinical infection would appear to be threatened by the potential development of resistance to last-resort vancomycin, a powerful antibiotic without deep backup. Thus, it is not yet clear that, even if we gain a detailed understanding of mutation in tumor-causing systems, we can devise general and widely applicable remedies for the range of diseases that fall under the cancer umbrella. However, without deeper insights into the operation of mutant-driven resistance, the opportunities for rational intervention are considerably diminished whatever the long-term prospects may be.