RATIONALE

The focus of this thesis has been four-fold. On one hand it has been to decipher, understand and manipulate the role of microtubule-trafficking in the cell. Secondly we have concentrated on the mechanism of action of agents that target the microtubules, and thirdly we have developed a model to explain acquired drug resistance to these microtubule-targeting agents. Lastly, we tested new microtubule-targeting agents that overcome acquired and intrinsic drug resistance to microtubule-targeting agents.

1. Microtubules (microtubules) are major dynamic structural components in cells that are essential for the development and maintenance of cell shape, cell signaling, movement, and division. In this study we seek to understand the role microtubules play within the cellular context. Our hypothesis is that microtubules act as "active highways" within the cells and are essential for the correct operation of the cell by controlling the delivery, location, and function of a plethora of proteins. We have focused on the p53 and the HIF1-α proteins. Both these proteins are crucial players in tumor progression and angiogenesis. p53 is a tumor suppressor gene commonly referred to as the guardian of the genome and HIF1-α is a transcriptional factor that plays a key role in adaptation to hypoxia. Upon DNA damage or hypoxia, p53 or HIF1-α respectively are induced and quickly localize to the cell nucleus. Whereas upon DNA repair or normoxia they must quickly localize to the cytoplasm for degradation. Our hypothesis is that their fast movement rate is not random and must be directed by a microtubule-driven motor.

2. Drugs that bind to either tubulin or microtubules form one of the most effective classes of anticancer agents. The so-called anti-mitotic drugs usually arrest cells in mitosis leading to apoptosis. In this study we analyzed the differential effects of taxol treatment on parental and taxol-resistant cells. Our hypothesis is that there must be a mechanism within the cell for sensing mitotic arrest and leading to apoptotic cell death. We were also interested in Laulimalide, a "new"

microtubule-targeting agent. Our goal was to characterize Laulimalide and classify it along with other microtubule-targeting agents.

3. Despite the clinical success of microtubule-targeting agents, the emergence of acquired resistance to the drug is a limiting factor for curing cancer. Acquired drug resistance is the most common reason for the failure of drug treatment in cancer patients with initially sensitive tumors, and as such, is presently responsible for the majority of deaths from cancer. In this study we sought to understand the timeline of events that takes place during the development of drug resistance to microtubule-targeting agents. While it has been widely published that a major mechanism of resistance to anti-mitotic drugs is due to acquired β-tubulin mutations, we hypothesized that another genetic event must occur to confer higher levels of resistance.

4. To overcome drug resistance to microtubule-targeting agents we have focused on alternate drug regimens that are active in anti-mitotic drug-resistant cells. The synergistic interaction of farnesyltransferase inhibitors (FTI) and taxol has recently been introduced in the clinic and surprisingly overcomes taxol resistance. In an effort to dissect the molecular mechanism underlying the synergistic interaction of FTIs with taxanes, we have recently discovered that FTIs affect microtubule acetylation and stability, partly due to inhibition of the tubulin deacetylase HDAC6. Thus, we hypothesize that inhibition of HDAC6 by the FTI lonafarnib leads to increased tubulin acetylation and that this is the molecular basis for the synergy of FTIs with Taxol.

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