A novel approach to cancer chemotherapy

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Richard F. Ludueña, Professor Emeritus at the University of Texas Health San Antonio, discusses his innovative approach to cancer chemotherapy, which could significantly enhance its effectiveness

The fundamental limitation to cancer chemotherapy is that cancer cells are our own cells misbehaving. Chemotherapy targets a protein or process that the cancer requires to survive and grow, but normal cells require the same protein or process to survive and grow, limiting chemotherapy's effectiveness. Also, cancer cells can mutate to become resistant. I propose a two-targeted approach that should harm only cells that require both targets. Most cancer cells fulfill these criteria, but most normal cells do not.

Microtubules, tubulin, and cancer chemotherapy

Microtubules are cylindrical organelles found in virtually all eukaryotic cells. They play critical roles in mitosis, intracellular transport, and cell motility. Microtubules comprise the protein tubulin, a dimer of two subunits: α- and β-tubulin. A key property of microtubules is their dynamicity: the rates at which α/β dimers assemble and disassemble. Microtubules are a major target of cancer chemotherapy; the tubulin-binding drug taxol has been useful. It works by 'freezing' microtubule dynamics, which causes a cell to die. Normal cells also require microtubules, however, so toxicity is a problem.

Tubulin isotypes

Both α- and β-tubulin exist as isotypes with different amino acid sequences encoded by different genes. Here, we focus on the β-tubulin isotypes. Each β isotype can participate in mitosis and most other microtubule functions; they differ in how well they perform these functions and their localization. For example, both βII and βIII are abundant in nerves, but βIII is only in neurons, while βII occurs in both neurons and glial cells. We have purified and studied the α/βII, α/βIII, and α/βIV dimers from cow brains.

In collaboration with Leslie Wilson and colleagues, we found that α/βIII forms very dynamic microtubules and that the dimers differ in their interactions with taxol, which interacts best with α/βII and least with α/βIII. These results can explain the effect of taxol on cancer cells as well as its side effects.

Cancer cells express large amounts of βII- and βIII-tubulin. βII may help them to rearrange membranes, as would happen to the nuclear envelope during cell replication, while βIII could promote growth and metastasis by making very dynamic microtubules. Also, neuronal microtubules have their dynamics constrained by microtubule-associated proteins (MAPs), so they may not be directly affected by taxol. Still, taxol kills the glial cells on which the neurons depend and thus causes neuropathy.

The first target: Nuclear βII-tubulin

Consuelo Walss-Bass discovered that βII-tubulin was present in the nuclei of certain cultured cells but not in microtubule form. Fluorescent α/βII microinjected into the cells localized to the nuclei but only after a cycle of cell division; in other words, the α/βII dimer did not penetrate the nuclear envelope; the nucleus re-formed around it. In contrast, α/βIII and α/βIV never went to the nucleus, so this is a phenomenon specific to βII. A study by I-Tien Yeh found that nuclear βII occurred in many different human cancers. Nuclear βII was also abundant in bone marrow and placenta, suggesting it was associated with dividing cells. The finding by Jiayan Guo that βII-tubulin was required for neurite formation in differentiating neuroblastoma cells supports the hypothesis that membrane rearrangements, including re-formation of the nuclear envelope after mitosis, require βII-tubulin. Anna Portyanko found that expression of βII-tubulin, especially nuclear βII, is associated with increased mortality of colorectal carcinoma patients, underlining the importance of βII-tubulin in cancer progression.

The second target: βIII-tubulin

Unlike βII-tubulin, which is fairly widespread, βIII-tubulin is abundant only in neurons. In addition to forming dynamic microtubules, βIII may protect cells from oxidative stress. This is particularly important for microtubules, whose formation can be inhibited by oxidizing agents. This property may also account for high levels of βIII in cancer cells, often under oxidative stress. βIII interacting poorly with taxol may explain why drug-resistant cancers express more βIII.

The combination attack: A role for CRISPR

I propose the following approach. First, one links the α/βII dimer to CRISPR-cas9 with a guide RNA for βIII. Injection of this into a cancer patient could allow this complex to localize to the nuclei of dividing cells, including cancer cells. If the guide RNA could block βIII expression, then the cancer cell would lose the advantages conferred by βIII, namely, highly dynamic microtubules and resistance to oxidizing agents. The cancer cell would still make βII, which interacts well with taxol; chemotherapy would thus be much more effective. Also, the cancer could not become resistant by making βIII.

What about cells that divide quickly, like bone marrow cells? The proposed complex could go to the nuclei of these cells, but bone marrow expresses little βIII, so it is unlikely that loss of βIII would harm it. What about normal cells that express large amounts of βIII, namely, neurons? These cells need βIII, but in adults, they rarely divide, so since the α/βII dimer localizes only to the nuclei of dividing cells, neuronal βIII would not be affected.

In short, this approach would only work on cells that divide quickly and make large amounts of βIII. Thus, cancer cells would constitute a likely target for this approach. However, normal cells also divide, albeit more slowly, and might even utilize a little βIII. How can this be dealt with?

Both α- and β-tubulins have an N-terminal methionine residue. This makes them very resistant to breakdown by the ubiquitin system. If the α/β dimer in our complex were synthesized without those methionines, they would both begin with arginine, which promotes degradation by the ubiquitin system. Other amino acids have intermediate affinities for ubiquitin. In other words, the α/βII-CRISPR-cas9 complex could be designed with an 'expiration date' so that it would only be effective for a limited period of time, remaining intact long enough to damage cancer cells but not long enough to damage normal cells.

Challenges for further experimentation

1. Joining the α/βII dimer with the CRISPR-cas9 complex without altering the relevant properties of either component. A suitable cross-linker could be found.
2. Having the complex enter the cell. A liposome or viral capsid may do the trick.
3. Cancer cells that do not over-express βIII may over-express βV instead. βV resembles βIII and may share some of its properties. If this is a serious problem and the proposed approach has yielded some positive results, one could envision synthesizing α/βII-CRISPR-cas9 with a guide RNA directed at βV.
4. Tissues whose cells multiply quickly are likely to require βIII. These would include the placenta and the growing brains of newborn children. Therefore, this method may not be suitable for treating cancers in pregnant women or young children.

Conclusion

Although the approach described above has not been tested, there is no reason to imagine that it would not work, and every reason to hope that it could be a successful therapy for cancer.

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