βII-Tubulin in Cancer: The Potential for CRISPR-based Oncology Treatments

Richard Ludueña
University of Texas Health Science Center

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Tubulin has biological significance beyond just microtubules. Professor **Richard Ludueña** of the University of Texas Health Science Center at San Antonio, has investigated the localisation of βII-tubulin in the nuclei of cancer cells. Based on his collaborators' insights, he speculates that βII-tubulin-based CRISPR-Cas9 treatments could become a cutting-edge treatment paradigm for a variety of different cancers.

Beyond Microtubules: β-Tubulin in the Nucleus

Tubulin is an essential 'housekeeping' protein in eukaryotic cells. The cell's cytoskeleton is supported by tubulin-based microtubules. Microtubules also provide structural support and play key roles in intracellular transport and the cell cycle. During cell division (mitosis), the microtubules form the mitotic spindle, which separates daughter chromosomes.

Tubulin is a heterodimer of two subunits – α and β. There are various isotypes of β-tubulin, encoded by different genes, with varying distributions across different tissues. βI- and βIV-tubulins predominate across a number of tissues. βV-tubulin is also widely distributed, though not as predominant. βVI-tubulin is mainly present in hematopoietic tissues, such as bone marrow and platelets. βII-tubulin is highly expressed in neurons and supporting cells such as glia, as well as muscles. βIII-tubulin is also common in the neurons (except glia), as well as testes.

When tissues become cancerous, their β-isotype expression profile changes profoundly. In particular, βII-tubulin is expressed in various cancers, and aggressive cancers also express βIII-tubulin. Professor Richard Ludueña of the University of Texas Health Science Center at San Antonio has carried out extensive research with a particular focus on oncologic βII expression.

In the cell's cytoplasm, microtubules are formed when α- and β-tubulins assemble (polymerise). However, Professor Ludueña's group has shown that this is not the only polymeric form of tubulin. In 1999, his then-graduate student, Dr Consuelo Walss, made a seminal discovery while studying cultured rat kidney mesangial cells. She found αβII-tubulin dimers as small bodies – possibly filaments – in the cells' nuclei, in both the nuclear matrix and nucleolus. This was surprising, as tubulin was normally thought to be present exclusively in the cytoplasm. This led to further questions – What was tubulin doing in the nucleus? How did it get there? Why, specifically, was the βII isotype there?

Dr Walss carried out further studies to answer these questions. She injected fluorescently labelled αβII, αβIII and αβIV dimers into rat kidney mesangial cells. A curious observation was that only αβII – not αβIII nor αβIV – entered the nucleus. Moreover, the αβII dimers only localised in the nuclei after a cycle of cell division. During mitosis, the nucleus disintegrates and later reforms. The findings suggested that the αβII dimer is not transported across the nuclear envelope; rather, the nuclear envelope reforms around it.

β-Tubulin in Cancer

Rat mesangial cells are a useful model system as they proliferate rapidly when cultured. But how do these findings translate to humans? Dr Walss carried out human cell culture studies, and found nuclear βII-tubulin present in all cancer cell lines tested but only a few normal cell lines. This was a Eureka moment – as nuclear βII was shown to be associated with cancer. This makes sense, given that common chemotherapy drugs, including vinblastine and taxol, arrest cell division by targeting tubulin-based microtubules and interact most strongly with αβII.

While studies involving cancer cell lines are a good starting point, it is important to investigate cancer tumours from patients. Professor Ludueña, therefore, collaborated with Dr I-Tien Yeh of his institution's Pathology Department. They immunohistologically analysed tumour tissues obtained from 201 patients with cancer to detect βII-tubulin. Three-quarters

(75%) of tumours expressed nuclear βII, while almost as many (72%) expressed cytoplasmic βII – but findings varied by cancer type. Over 8-in-10 epithelial cancer samples (83%) tested positive for nuclear βII, compared with just half (54%) of non-epithelial cancers. Nuclear βII was present in all colon and prostate cancers, often highly expressed. In contrast, nuclear βII was infrequently present in skin cancer (19%). However, intra-tissue distribution often varied within samples. Notably, nuclear βII was often present in 'normal' tissue adjacent to tumours.

These findings suggest that as cells become cancerous, they begin to make βII-tubulin and localise it in their nuclei. What's more, tumour cells somehow seem to induce neighbouring cells to do the same, and it seems that nuclear βII serves an unknown function for cancer cells. Potentially, βII may be involved in cancer cell signalling. Dr Ludueña's then-graduate student, Jiayan Guo, found that silencing βII in cultured neuroblastoma cells inhibited the development of neurites, suggesting that βII may play a role in membrane rearrangements, which may be involved in cancer cell migration. Professor Ludueña believes it is important to identify components of this pathway, and this could drive the development of new anti-tumour drugs that inhibit these components.

Does nuclear localisation of βII-tubulin influence cancer outcomes? A study by Professor Ludueña's collaborator, Dr Anna Portyanko, at Belarussian National University, suggests a correlation. Her group followed up 124 patients with colorectal cancer and analysed their tumour biopsies. They found βII-tubulin (in cytoplasm or nuclei) in almost one-third of patients (28%), of which nearly half had nuclear βII-tubulin (11% of the total cohort). Tumours are composed of a centre and an invasive edge (at the interface with normal tissue). βII-tubulin was almost three times more likely to be detected exclusively in the tumour centre than exclusively in the invasive edge (17 cases vs. 6 cases). Patients with βII-tubulin in the tumour had a reduced life expectancy than those without, and this was even shorter in patients with nuclear βII.

These studies suggest that nuclear βII-tubulin detection could be used for more accurate diagnosis of certain cancers. Of particular significance is the detection of βII outside the tumour itself. If a biopsy probe misses the tumour itself, normal cells surrounding the tumour may test positive for βII – either nuclear or cytosolic. This would not only avoid a false negative but could lead to an earlier diagnosis and indicate disease state. Dr Portyanko's study demonstrates the prognostic implications, suggesting that βII-tubulin could be used as a biomarker to predict survival outcomes.

CRISPR'ing with βII-tubulin

CRISPR-Cas9 (informally known as CRISPR) is a gene editing tool with promising medical applications. Many hereditary diseases are caused by gene mutations – these may be 'corrected' using CRISPR-Cas9. In oncology, the preferred approach is to use CRISPR-Cas9 to arrest genes involved in cancer. CRISPR-Cas9 is composed of two components. A guide RNA (gRNA) targets a specific gene sequence and provides the 'edited' nucleotide code. A Cas9 (CRISPR-associated protein 9) endonuclease enables the edit to take place. The potential of CRISPR-Cas9 for treating cancers is worth pursuing. Professor Ludueña had a smart idea. It occurred to him that CRISPR-Cas9 could be combined with βII-tubulin. This could enter the nuclei of cancer cells and arrest essential genes, preventing further tumour proliferation or hastening cell death.

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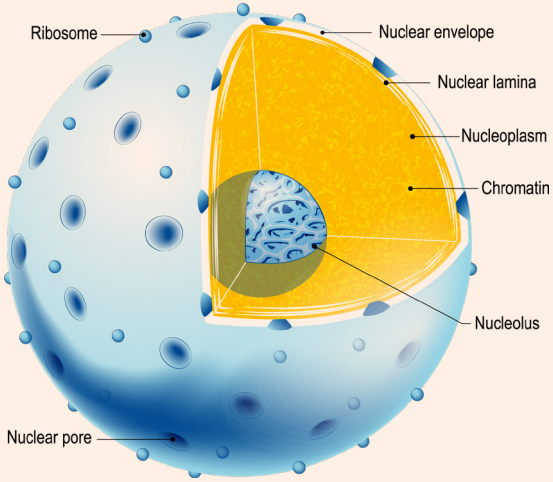
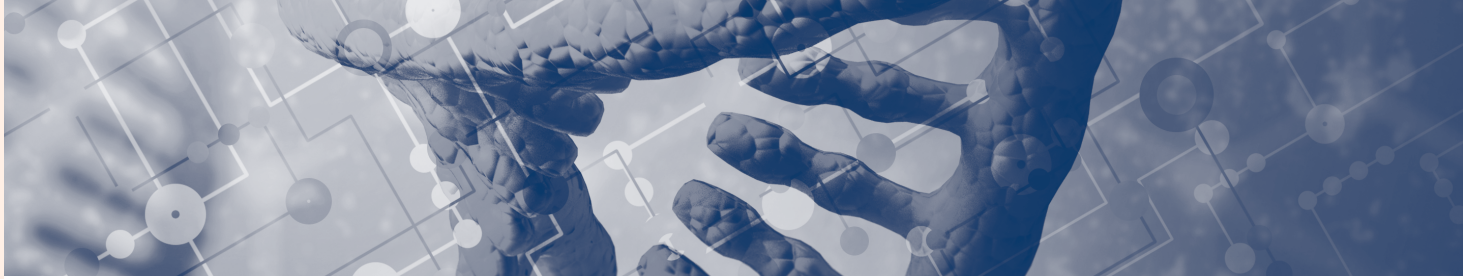
CELL NUCLEUS





There are a number of questions that Professor Ludueña believes must be addressed before a βII-CRISPR-Cas9 complex becomes a feasible cancer therapy. Firstly, how would βII-CRISPR-Cas9 enter cancer cells? It may be possible to add a receptor-binding factor, target CD20 cell surface proteins, or encapsulate the complex in an adeno-associated virus. Secondly, how would βII-CRISPR-Cas9 enter into the nucleus? Professor Ludueña suggests that βII-tubulin should be complexed to α-tubulin – this should enable the βII-tubulin to maintain its functional conformation. In principle, no nuclear recognition signal would be required for the αβII-CRISPR-Cas9 complex to enter the nucleus. The nucleus is expected to re-assemble around the complex after mitosis.

The third question is, what would the gRNA target? The gRNA could target 'housekeeping' genes, such as those involved in sugar or energy metabolism, or cell reproduction, such as DNA polymerase. The tumour cells would then be unable to respire or divide – thus arresting cancer development. However, this could lead to negative side effects, since normal cells may need these same proteins in order to function and reproduce.

Professor Ludueña suggests that, alternatively, gRNA could target the βIII-tubulin isotype gene – as cancer cells often express both βII and βIII. This may limit unwanted effects on normal cells, as only neurons and testes normally express βIII-tubulin in significant quantities. Neurons rarely divide, if ever, in adults, limiting nuclear localisation. The testes may be affected, and the worst result would be male infertility (a non-lethal side-effect of many anti-tumour drugs), but this would not affect women. This could be an option for female-specific cancers, such as ovarian, gynaecological, or breast cancers. If the cancer cell is unable to produce βIII, it would be less resistant to drugs such as taxol and vinblastine since these drugs interact relatively poorly with βIII. Normal cells that rapidly divide, such as bone marrow, produce little or no βIII, so it is envisaged that little harm would ensue. However, caution should be exercised when proposing such a treatment for pregnant women, infants, and children, as their cells divide more rapidly.

There are several challenges to be overcome if αβII-CRISPR-Cas9 is to be used in cancer therapy. α- and βII-tubulins, linked to CRISPR-Cas9, must be stable and maintain their conformations. A major safety consideration is to ensure that αβII-CRISPR-Cas9 acts on the tumour cells and not normal cells (some of which also produce βII). This could be achieved by including a 'self-destruct' mechanism in αβII-CRISPR-Cas9. Professor Ludueña speculates that αβII-CRISPR-Cas9 could be fine-tuned, for example, by removing the N-terminal methionine from the α- or βII-tubulins. This would allow it to be degraded by the cell's ubiquitin system. As αβII only seems to enter the nucleus with mitosis, and normal cells divide more slowly than cancerous cells, this could remove αβII-CRISPR-Cas9 from normal cells before it can damage their DNA. Another challenge relates to αβII-CRISPR-Cas9 treatments that target βIII-tubulin. βV is similar to βIII, and tumours may develop resistance to βIII-silencing therapies by over-expressing βV. A possible solution would be to have a second αβII-CRISPR-Cas9 with a gRNA targeting βV or a backup chemotherapy that targets βV.

Despite the inevitable challenges ahead, Professor Ludueña's research into nuclear βII-tubulin expression in cancer has important diagnostic, prognostic, and therapeutic implications. His idea – combining αβII with CRISPR-Cas9 – could represent a new cutting-edge therapeutic paradigm for a variety of cancers.

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MEET THE RESEARCHER

Professor Richard F Ludueña, Department of Biochemistry and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Professor Richard Ludueña is an Emeritus Professor in Biochemistry. His research focuses on the biochemistry, molecular biology, and the therapeutic implications of tubulin proteins, particularly in the development of new anti-tubulin anti-tumour drugs. Having obtained a BA in Chemistry at Harvard in 1967 and a PhD in Biological Sciences at Stanford in 1973, he continued his postdoctoral training at Stanford. In 1976, he embarked on an Assistant Professorship at the University of Texas Health Science Center at San Antonio, where he remains to this day, having risen to the esteemed rank of Emeritus Professor. Over the years, he has taught various modules, including medical biochemistry, cell biology, and drug design and discovery, and has won numerous teaching awards. He is a prolific author, and he has published over 160 papers, as well as two textbooks consisting of biochemistry-related library projects for medical students.

CONTACT

luduena@uthscsa.edu

www.researchgate.net/profile/Richard-Luduena

KEY COLLABORATORS

Professor Consuelo Walss-Bass, University of Texas Health Science Center at Houston

Dr Anna Portyanko, Belarusian State Medical University, Minsk

Dr Kseniya Ruksha, Belarusian State Medical University, Minsk

Dr Jiayan Guo, Fosun Pharma, Shanghai

Professor I-Tien Yeh, University of Texas Health Science Center at San Antonio

Dr Asok Banerjee, University of Texas Health Science Center at San Antonio

Dr Israr Khan, University of Texas Health Science Center at San Antonio

Dr Brent Derry, University of California, Santa Barbara

Dr Veena Prasad, University of Texas Health Science Center at San Antonio

Dr Leslie Wilson, University of California, Santa Barbara

Dr Mary Ann Jordan, University of California, Santa Barbara

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FURTHER READING

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