Structure and functions of microtubules

Describe the Structure and Functions of Microtubules – Why Can Drugs That Interfere With Microtubule Assembly Be Used as Cancer Therapy?

Introduction

Microtubules form as a highly organised network of polarised tube filaments from a protein called tubulin. Its regulation is needed for processes such as mitosis, cell migration, cell signalling and cell trafficking. The microtubules themselves are regulated by several kinases and phosphatases via signalling cascades, and concomitantly by interactions with actin cytoskeleton and adhesion sites.

Microtubule-targeted drugs (MTDs) constitute a major anticancer therapeutic class having properties of anti-mitotic and anti-angiogenic properties, thereby inhibiting malignant cell growth mainly by altering microtubule dynamics in both cancer and endothelial cells. The key to design of MTDs and the understanding of tumour progression regulators is the identification of proteins regulating the microtubule network.

Cell Morphology and Function

As the name implies, microtubules are hollow tubes having an external diameter of roughly 25nm and a cell wall thickness of 5-7nm. One of their functions is to transport organelles (e. g. secretetory vesicles) through the cytoplasm, a particularly important role in nerve cells where axoplasmic flow is required. They also have a critical role in cilia and flagella movement. Microtubules originate from a complex structure known as centrosome.

Between cell divisions (figure 1), the centrosome is located at the centre of a cell near the nucleus. Embedded in the centrosome are two cylindrical

centrioles, arranged at right angles to each other. At the onset of cell division, a centrosome divides and the two daughter centrosomes move to opposite poles of the nucleus to form a mitotic spindle

The functions of microtubules are important to the survival of eukaryotic cells because, along with actin and intermediate filaments, microtubules constitute the cytoskeleton which offers shape and strength to the cytoplasm. It is therefore vital that we understand their fundamentals, such as what they are composed of and how their structure is both maintained and destroyed within cells.

As mentioned in the introduction, the building blocks microtubules are tubulin. However, only two forms of tubulin, α-tubulin and β-tubulin, play a role in the formation of the microtubule structure. When the α and β-tubulin bind, a useful subunit called a heterodimer forms.

When intracellular conditions favour assembly, tubulin heterodimers assemble into linear protofilaments, which in turn assemble into microtubules. All such assembly is subject to regulation by the cell. [11]

The interactions holding α and β-tubulin in a heterodimeric complex are strong enough that α tubulin subunit rarely dissociates under normal conditions. Each tubulin subunit binds two molecules of GTP. One GTP-binding site, located in α-tubulin, binds GTP irreversibly and does not hydrolyze it, whereas the second site, located on β-tubulin, binds GTP reversibly and hydrolyzes it to GDP. The second site is called the exchangeable site because GDP can be displaced by GTP. The recently solved atomic structure of the tubulin subunit reveals that the non-

exchangeable GTP is trapped at the interface between the α and β-tubulin monomers, while the exchangeable GTP lies at the surface of the subunit

Microtubules may appear to be stable but they usually oscillate between growth and shortening phases. During growth, heterodimers are added on to the end of a microtubule, and during shrinkage they come off as intact subunits. [11]

This active process of assembly and disassembly can be inhibited by a range of drugs that bind to many sites in the β-tubulin subunit. These drugs prevent mitotic division and ultimately lead to cell death, by means of necrosis and apoptosis.

The subunits are aligned end to end into a protofilament. The side-by-side packing of protofilaments forms the wall of the microtubule. In this model, the protofilaments are slightly staggered so that a-tubulin in one protofilament is in contact with a-tubulin in the neighboring protofilaments.

Microtubules and Cancer Therapy

The aim of treatment in patients with cancer is cure or, if this is not possible, effective palliation of many cancers present as localised tumour masses, but surgery or radiotherapy often fails to eradicate the disease, which eventually becomes widespread. For this reason, there is a trend to incorporate systemic treatment with local treatment at the time of diagnosis. [3] The basic mechanism of anticancer drugs is the inhibition of cell proliferation processes.

However if they fail to selectively target tumour cells over proliferating normal cells, this renders the drug toxic. This particularly arises in the bone marrow, gastrointestinal epithelium and hair follicles. A cytotoxic drug is said to be selective in cancer therapy if it inhibits malignant composite cells undergoing division and concomitantly allows for normal cell proliferation.

Anticancer drugs are classified according to their sites of action either during the cell cycle or along the synthetic pathway of cellular macromolecules. Some drugs are only effective during part of the cell cycle, which are termed phase-specific drugs, while others are cytotoxic throughout the cell cycle usually called cycle-specific drugs [3].

Vinca alkaloids and taxanes are drugs that inhibit mitosis by binding to the microtubular proteins necessary for spindle formation. They can therefore be classified as phase-specific drugs – to be more precise however; they are M-phase specific.

The main vinca alkaloids are vincristine, vinblastine and vindesine. They have been used in the treatment of cancer for over many years. It is because of their efficacy that has guaranteed that they remain among the drugs of choice for numerous types of human cancers, “ They are used in acute lymphoblastic leukaemia, lymphomas and some solid tumours” [3]. They bind to tubulin and inhibit its polymerisation into microtubules which prevents spindle formation. [14]

Microtubules are central to a number of cellular processes including the formation of the mitotic spindle. It is without a doubt that the destruction of

the spindle leads to a loss of chromosome segregation which consequently inhibits cell division causing cell death.

Although the cell spindle was an obvious goal for further drug development, research in this area was obsolete until the exciting clinical results of the taxane drugs were reported in the late 1980s [4].

The taxanes paclitaxel and docelatel also bind to tubulin, however these agents, in contrast to the vinca alkaloids, stabilise the spindle and produces mitotic arrest. Thus, microtubule stabilisation leads to similar effects as microtubule disruption.

Research in the field has again increased following the observation that agents that bind to tubulin can selectively destroy the arrangement of blood vessels within tumours, causing widespread tumour necrosis [5]. It may be possible that the vinca alkaloids and the taxane drugs also exert part of their tumour-destroying action through an antivascular mechanism. This may depend on targeting endothelial cells rather than tumour cells for drug action. A possible advantage of this approach is that endothelial cells are not transformed and are unlikely to acquire mutations resulting in drug resistance. [6] The destruction of the tumour vasculature also arises through a tubulin-related mechanism.

The disorder of the microtubular arrangement impairs the cell function because microtubules are involved in the maintenance of cell shape. The tumour selectivity begins from the unsystematic character of tumour blood vessels. Rouget cells or pericytes are associated abluminally with all vascular capillaries and post-capillary venules. [10] The tumour blood vessels lack

these cells along with sustaining smooth muscle which causes them to be feeble.

Therefore the endothelial cells lining the tumour blood vessels are more vulnerable to the effects of vinca alkaloids and taxanes. Although contact with these anticancer drugs is experienced by all vascular endothelial cells, it is the vulnerable tumour blood vessels that are damaged the most. This ultimately leads to necrosis of tumour cells that were reliant on the blood vessels.

One problem seen in these studies is the survival of cancer cells at the periphery of the tumour [7]. These are nourished from the blood vessels of the normal neighboring tissue and are therefore not affected by the damage of blood vessels in the tumour. These tumour cells are likely to increase in number again. For that reason, it is doubtful that these anticancer drugs will be effective unless given in combination with additional therapies. This may be strikingly more successful than single drugs, for example in the treatment of some cancers such as Hodgkin’s disease. [3]

The shortcoming in previous clinical trials on agents targeting tubulin was the rejection of potentially useful agents because interest was more centered on toxicity and survival of drugs, rather than the action or effects of drugs on blood vessels. The breakthrough of new antivascular treatments would be an essential addition to cancer therapy; hence it is these agents that are presently most fascinating to scientists.

Other Drugs That Inhibit Function of Microtubules

There are more than thirty drugs in the past or in present clinical development. [13] In order to maintain a reasonable size for the following sections, only a few of the more fascinating drugs will be discussed. Some that where not mentioned previously include:

- Taxol – an anti-cancer drug, stabilises microtubules
- Colchicine – binds tubulin and blocks polymerisation. Microtubules depolymerise at high colchicine concentration.
- Nocodazole – causes de-polymerisation of microtubules.
- Actinomycin – antibiotic able to halt cancer, not widely used as it is highly toxic

The Microtubule Network as a Target for Therapeutic Agents

The various M-phase specific drugs act by targeting different parts of the heterodimer. To date, three binding areas have been acknowledged: the colchicine site close to the α/β interface, the region where the vinca alkaloids bind, and the taxane binding pocket. [13]

Colchicine, currently a medication for acute gout, also inhibits cell division and has therefore previously been used in cancer therapy. It binds to a site near the α and β-tubulin interface within the microtubule, blocking microtubule polymerisation [15]. However, its high toxicity prevents its use for current cancer therapy.

Vinca alkaloids inhibit microtubule assembly by cross-linking at the inter-dimer interface; they sterically distort the protofilament and induce tubulin to form alternate spiral polymers [16].

The mechanism of action of taxanes is quite different from that of the other two, for it promotes the assembly of microtubules, resulting in highly stable, non-functional polymers. Taxanes bind at the M loop on the β-subunit, stabilising lateral contacts between protofilaments [17].

Antimitotic agents that interact with microtubule components are of interest for the insights they can provide into the roles of microtubules in cells and the subtleties of tubulin structure and also for their potential activity in the treatment of human neoplastic diseases. A variety of bioassays have been used to identify new antitubulin agents and new techniques have been developed to further understand their biological potency and mechanistic basis at the molecular level.

Drug Combinations

Although M-phase specific drugs are remarkable in that it prevents further malignant growth, the administration of combinations of drugs given intermittently often produces better results than more continues treatment with a single drug. The rationale is that a combination of drugs with different toxic effects and affecting different biochemical pathways has anti-tumour activity without addictive toxicity. [3]

However, a large number of antimitotic drugs are currently under development, this implies that microtubules are still a very worthwhile target for anticancer therapies.

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