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Cytoskeleton Analysis as Target for Bioactives

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ABSTRACT

Cytoskeletons are the proteins contained within the cytoplasm and responsible for many cellular and physiopathological events such as maintenance of cell shape, chromosome aggregation, mechanical support to cells, muscle contraction, provide mechanical strength etc. Cytoskeletons are of three major types: Microfilaments, intermediate filaments and microtubules. They are collectively responsible for providing most of the structure and spatial organization in the cell. In this review, we focus on the drugs, bioactive and other active agents which act on the cytoskeletal elements and also emphasize on the method used to visualize or analyze the cytoskeleton structures and elements. Different classes of cytoskeleton acting drugs, agents, their importance, target area and their mode of action are also focused in this review with the contribution of the different instruments for the analysis of cytoskeleton types which are important for the drug targeting.

Key words: Cytoskeleton, cytoplasm, microfilaments, intermediate filaments, microtubules, bioactives

INTRODUCTION

The cytoskeleton is cellular supporting framework or skeleton contained within the cytoplasm and is mainly consisted of proteins. This dynamic structure control the cell shape, protects the cell, enables cellular motion and plays important roles in both intracellular transport (the movement of vesicles and organelles) and cellular division (Janmey, 1995; Ramaekers and Bosman, 2004). highly complex. Cytoskeletons are flexible and dynamic structure involved in a variety of cellular functions essential for cell survival including motility, maintenance of cell shape, cell attachment and interaction with the extracellular matrix (i.e., through focal adhesions) anchorage of cell organelles and maintenance of cytoplasmic viscosity. The cytoskeleton is composed of three types of cytosolic fibers: Microfilaments, intermediate filaments and microtubules and arranged in a complex network (Woll *et al*., 2005).

Analysis of the various cytoskeletons and cytoskeleton proteins play very important role on the drug targeting. Different types of analytical instruments are used for the analysis of cytoskeleton elements and their role on the different function and diseases. Commonly used instrument for cytoskeleton analysis are flow cytometry (Woll *et al*., 2005; Nebe *et al*., 1997), indirect immunofluoroscence (Marcum *et al*., 1978), electron microscopy (Small *et al*., 1999), tubuline assembly assay (Himes *et al*., 1976; Grosios *et al*., 1999), sedimentation assay (Combeau *et al*., 2000), confocal microscopy (Dailey *et al*., 2006; Claxton *et al*., 2006), fluorescent spectrophotometer (Damania *et al*., 2010; Sattelle, 1988), competitive binding assay (Hadfield *et al*., 2003), cytoblot

assay (Chiosis and Keeton, 2009) and mass spectrometric analysis (Sakamoto *et al.*, 2008) which are very useful in the determination of action of drugs and also helpful to observation of cellular and structural changes.

As a major, ubiquitous protein in all metazoan cells, actin serves central roles in shape determination, cytokinesis and cell motility, as well as in the establishment of cell-cell and cell-matrix interactions (Da Costa *et al.*, 2003). A notable property of living cells is their ability to regionally control the polymerization and supramolecular organization of actin filaments, involving the engagement of a broad spectrum of actin binding proteins and resulting in the formation of different structural sub compartments, each with a defined function. In epithelial cells, a circumferential band of actin filaments provides the structural support for cell-cell junctions; in motile cells, such as leukocytes, actin filaments form the meshed framework of the protruding lamellipodia and linear filopodia and immobile fibroblasts are anchored flat to the underlying matrix via Trans membrane coupling to linear bundles of actin filaments, or stress fibers. In response to external signals, dramatic changes in cell shape and motility can be effected and all involve the controlled and dynamic reorganization of the actin cytoskeleton (Amos and Amos, 1991).

The interplay between the three-dimensional protein network called the cytoskeleton and the two-dimensional lipid bilayer which forms the cell membrane is a central feature of cell biology and a richly complex physical and chemical phenomenon. The complexity of the membrane/cytoskeleton boundary derives in part from the intricacy of the interface between two soft materials and in part from the number of distinct molecules and chemical interactions that occur at this interface and influence its physical properties and chemical composition. The importance of the field and the volume of contributions to it have motivated many reviews (Small *et al.*, 1999).

The cytoskeleton spans the cytoplasm and interconnects the cell nucleus with the extracellular matrix, thereby forming a structural link between molecules involved in cell communication on the one hand and gene expression on the other (Erickson, 2007; Frixione, 2000). Since the cytoskeleton is involved in virtually all cellular processes, abnormalities in this essential cellular component frequently result in disease. In this introduction, the basic structure of the cytoskeleton is briefly outlined. Furthermore, the disease processes in which the cytoskeleton plays a decisive role (Luna and Hitt, 1992; Janmey, 1995). Sako *et al.* (2010) measured directly through the technique of micromanipulation by micropipette the viscosity of mouse muscle cells modified by transfection with the αβ-crystalline in order to highlight the role played by the changes in the cytoskeleton. Two vectors were used, the αβ-crystalline of wild type and R129G αβ-crystalline mutant. (Sako *et al.*, 2010).

COMPOSITION OF CYTOSKELETONS

In the diversity of interactions occur between membrane constituents and all three components of the cytoskeleton. The binding of the major cytoskeleton fiber proteins themselves (except for some intermediate filaments) to phospholipids appears generally to be relatively weak and transient (Resch *et al.*, 2002). Linking of cytoskeletal filaments with directly to the lipid bilayer, intact cells assemble complexes of various proteins at points where the cytoskeleton attaches to the membrane. Some components in these linkages span the lipid bilayer, some penetrate into the cytoplasmic face of the bilayer, some bind preferentially to specific phospholipid head groups and others bind cytoskeletal filaments either directly to the lipid bilayer or indirectly to proteins bound to the membrane (Magin *et al.*, 2004). Types of cytoskeletons include: Microfilaments, Microtubules and Intermediate filaments. Comparison of various types of cytoskeletons have been shown in Table 1.

Table 1: Comparison of cytoskeleton types

Cytoskeleton type	Diameter (nm)	Structure	Subunit example	References
Microfilaments	6-8	Double helix	Actin	Bijman *et al.* (2008)
			• Vimentin	
			• Glial fibrillary acidic protein	Heng and Koh (2010)
Intermediate filaments	10-12	Two antiparallel helices/dimers, forming tetramersa	Keratin	Wagner *et al.* (2007)
			Nuclear lamin	Parry *et al.* (2007)
Microtubules	23-25	Protofilament, in turn consisting of tubulin subunits	Alfa and beta tubulin	Hadfield *et al.* (2003), Hemphill *et al.* (1992), Vieira *et al.* (2008)

Microfilaments

Composition: Actin filaments are globular protein and Globular monomer (G-actin) polymerizes into Filamentous (F-) actin which appears two right-handed helices wound around each other with a repeat distance of approximately 36 nm. Actin network plays an important role is the separation of centrosomes and thus very important for the drug which act during the cell cycle. Actin filaments with the microtubule and intermediate filament contributing to the morphological framework of a cell and which participates in the dynamic regulation of cellular functions (Heng and Koh, 2010).

Actin filaments have a diameter of 7 nm and can reach lengths of 30-100 μm *in vitro* and at least several microns *in vivo*. Because these filaments are so long, they form semi-dilute solutions at extremely low volume fraction (<0.05%) in which rotational diffusion of the filaments is greatly retarded due to solute-solute interactions. Nearly all mechanically relevant properties of actin filaments-length, stiffness, concentration, lateral or orthogonal aggregation can be regulated by one or more of scores of actin binding proteins found in the cytoplasm of most cells (Magin *et al.*, 2004; Schaub *et al.*, 2007).

Functions

- Regulation of actin cytoskeletal organization may have a crucial role in signaling lens cell differentiation (Bijman *et al.*, 2008)
- Actin bundles support projections of cell membrane (Lambrechts *et al.*, 2004)
- Change of shape of platelets during blood clotting (Lambrechts *et al.*, 2004)
- Actin polymerization act important role in toxins action (Small *et al.*, 1999)
- Give mechanical support to cells and hardwire the cytoplasm with the surroundings to support signal transduction (Svitkina *et al.*, 2007)
- In muscle cells, to be the scaffold on which myosin proteins generate force to support muscle contraction (Woll *et al.*, 2005)
- In non-muscle cells, to be a track for cargo transport myosins (non-conventional myosins) such as myosin V and VI. Non-conventional myosins use ATP hydrolysis to transport cargo, such as vesicles and organelles, in a directed fashion much faster than diffusion (Heng and Koh, 2010)

Microtubules

Composition: Microtubules are composed of α/β tubulin heterodimers units which are polymerized to form hollow cylinders the length of cylinder is approximately 25 nm diameters which can be more than 100 μm long. The walls of the microtubule are composed of 5 mm diameter linear proto filaments arranged in parallel. Like actin, the tubulin subunits bind nucleoside triphosphates and

hydrolyze them after they polymerize. Unlike actin which binds most purine nucleotides, tubulin binds GTP rather than ATP (Hemphill *et al*., 1992) microtubule form intracellular lattice like structure which is reorganized into the mitotic spindle (Risinger *et al*., 2009).

Functions

- Microtubules act as conveyer belts inside cells (Jordan and Wilson, 2004)
- They help to move vesicles, granules and organelles like mitochondria and chromosomes via special attachment proteins (Combeau *et al*., 2000)
- Microtubules also play a role in maintaining the cytoskeleton, that is, the basic structure of the cell because, structurally, they are linear polymers of tubulin which is a globular protein present in the cytoplasm (Tian *et al*., 2010)
- During non cell division, it organizes the cytoplasm, position of nucleus and organelles (Marcum *et al*., 1978; Himes *et al*., 1976)
- Microtubule provides shape and strength of cytoplasm (Hait *et al*., 2007)

Intermediate filaments

Composition: Intermediate Filaments (IFs) are thicker as compared to microfilaments and where as they are thinner than microtubules thus they are known as intermediate in them with respect to size (Godsel *et al*., 2008). They have approximately 10 nm in diameter and length in microns, in cells often ranging from the plasma membrane to the nucleus (Parry *et al*., 2007). They are responsible for the viscoelastic networks at low protein concentration *in vitro* and thus can be believe for provide mechanical strength to the cell *in vivo*. Intermediate filaments differ from microfilaments and microtubules in several respects (Herrmann *et al*., 2007). Unlike actin and tubulin which have only a few closely related isoform in the same species and are very strongly conserved in evolution, intermediate filaments are formed from polymers of proteins which varies according to cell types of the same species and between different species (Kreplak *et al*., 2005). The nature of intermediate filament proteins can be either acidic or basic. They may be present or may not be present in the cell. There are various unicellular eukaryotic cells which have the actin and tubuline filaments but lack of the intermediate filaments (Godsel *et al*., 2008). Intermediate filaments are very important for living cells and it is essential for the cell structure and function because they provide mechanical support and physical resilience for cells and tissue (Kim and Coulombe, 2007).

Functions

- Provide mechanical strength (Kim and Coulombe, 2007)
- Into the cytoplasm where they provide a scaffold for mitochondria, the Golgi complex, Microtubule Organizing Centers (MTOCs) and other cytoskeletal elements (Herrmann *et al*., 2007)
- In the periphery IFs associate with plasma membrane specializations such as desmosomes, hemidesmosomes and focal adhesions (Godsel *et al*., 2008)
- Play important role in cell signaling, growth, epithelial polarity, wound healing and apoptosis in addition to providing the cell with resilience to environmental stress (Parry *et al*., 2007; Goldie *et al*., 2007)
- IFs contribute the tensile strength necessary for maintaining cell integrity (Kreplak and Fudge, 2007)

THE MICROTUBULE NETWORK AS A TARGET FOR THERAPEUTIC AGENTS

Microtubules are the dynamic structure that are involved in various cellular processes, cell division, cell cycle and cell proliferation. They are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signaling and in cell division and mitosis. Agents or drugs which are act on the microtubules or tubuline subunits are affect all the above described cellular processes and thus they have very useful in the treatment of cancer therapy. Microtubules and their dynamics are the targets of a chemically diverse group of antimitotic drugs. Drugs and agents which are targeting into the microtubules are dividing into two classes first which are inhibit tubulin polymerization and second which are promote tubuline polymerization. Different tubuline targeting agents are summarized into the Table 2 and the importance of microtubules in drug targeting is summarized in the Fig. 1 (Rovini *et al*., 2011; Risinger *et al*., 2009). The polymerization of microtubules occurs by a nucleation elongation mechanism in which the relatively slow formation of a short microtubule nucleus is followed by rapid elongation of the microtubule at its ends by the reversible, non-covalent addition of tubulin dimmers (Hadfield *et al*., 2003).

Actin-targeted drugs: Actin microfilament is globular protein in which the G-actin monomers are polymerizes into filamentous network. Actin cytoskeleton involved in various type of cellular function such as cell movement, muscle contraction etc. A variety of drugs and toxins have been act on to the actin cytoskeleton and also they have been used to investigate the cellular role of actin. Generally the drugs and toxins which act with actin cytoskeleton may stabilize, depolymerize, polymerizes or rearrangement of F-actin filaments which are responsible for the changes of cellular function and other structural modification and thus actin are play important role on the drug targeting (Lambrechts *et al*., 2004). Actin targeted drug are divided into three major classes includes cytochalasins, latrunculins and jasplakinolides. Actin targeted drugs are summarized in the Table 3.

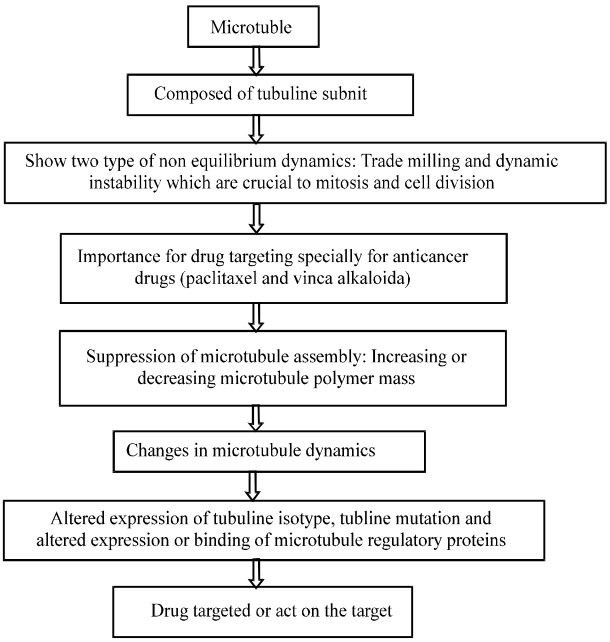

Fig. 1: Summary of importance of microtubule on drug targeting

Table 2: Agents which interact with microtubules

Groups	Binding domain	Sources	Mechanism of action	Related drugs or analogues	Therapeutic use	References
Microtubule destabilizing agents	Vinca domain	Leaves of periwinkle plants (*Catharanthus roseaus* belonging to family Apocynaceae)	Depolymerizes the microtubules at high concentration, generally acts on β-subunits of tubuline	Vinblastin	Hodgkins diseases	Lobert *et al.* (2007), Pereira *et al.* (2010)
				Vincristin	Leukemia, lymphomas	Ozgen *et al.* (2003), Wang *et al.* (2011), Kushner *et al.* (2011)
				Vinorelbine	Solid tumors	Zeybek *et al.* (2011) Viñolas *et al.* (2011)
				Vinflunin	Lung, breast cancer	Rovini *et al.* (2011), Hait *et al.* (2007)
				Cryptophycin 52	Solid tumors	Attard, *et al.* (2006), Risinger *et al.* (2009)
				Dolastatin	Potential vascular targetings agents	Hait *et al.* (2007), Risinger *et al.* (2009)
	Colchicines domain	*Colchicum autumnale* belonging to family Liliaceae	Inhibit microtubule polymerization by binding to microtubule ends	Colchicines	Non neoplastic diseases (gout, family Mediterranean fever)	Attard *et al.* (2006), Risinger *et al.* (2009) Babu *et al.* (2011)
				Combretastatins	Potential vascular targeting agents	
Microtubule stabilizing agents	Taxane domains	*Taxus bravifolia* belonging to family Taxaceae	Stabilize the microtubules and increases microtubule polymerization	Paclitaxel	Ovarian, breast and kaposis sarcoma	Yao *et al.* (2011), Elstad and Fowers (2009)
				Docetaxel	Prostate, lung and brain tumors	Liu *et al.* (2008), Mugabe *et al.* (2011)

Table 3: Agents which interact with actin

Class	Sources	Mode of action	Therapeutic use	References
Cytochalasin	Mold metabolites	Bind to barbed end of actin filament to prevent actin filament assemblt and disassembly means inhibition of microfilament polymerization	Fibrinogen and fibrin polymerization disorders, inhibit basal and insulin-stimulated glucose transport	Hayduk and Lee (2005)
Latrunculines	Red sea sponges	Bind to actin monomer and inhibit polymerization thus promotes filament disassembly	Antiangiogenic, antiproliferative, antimicrobial and anti-metastatic activities	Khanfar *et al.* (2010) Sattler *et al.* (2011),
Jasplakinolides	Marine sources	Stabilize actin monomer thereby enhancing filament nucleation and assembly	Antifungal agents	Scott *et al.* (1988), Visegrady *et al.* (2004)

Novel drug delivery systems for targeting to cytoskeleton: Cytoskeleton is dynamic structure, that controls the cell shape and involve many cellular function and cell division. The use of novel controlled delivery systems could be very helpful for the targeting of cytoskeletons (Khan, 2001) (Table 4). Different carrier system like liquid crystals (e.g., niosome, cubosome), lipid

Table 4: Analysis technique and their target area

Technique	Target area	References
Flow cytometry	Detection of cell cycle by measuring the DNA content	Woll *et al.* (2005), Nebe *et al.* (1997).
Indirect immunofluoroscence	Determination of intracellular distribution of microtubule	Small *et al.* (1999), Iwamoto and Tamura (1988)
Electron microscopy	Increase in turbidity is measured during polymerization experiment	Sakamoto *et al.* (2008)
Tubuline assembly assay	During the polymerization turbidity increased which is measured by spectroscopic method	Himes *et al.* (1976), Grosios *et al.* (1999)
Sedimentation assay	Mesuring the quantity of microtubule during the tubuline assay	Combeau *et al.* (2000)
Confocal microscopy	Direct measurement of cell shape	Small *et al.* (1999), Dailey *et al.* (2006)
Fluorescent spectrophotometer	Determinations of cell shape and size changes of cytoskeleton by light scattering	Small *et al.* (1999), Sattelle (1988), Damania *et al.* (2010) and Johnson *et al.* (1980)
Competitive binding assay	Antitubulin agents to compete with (3H)-radiolabelled colchicine, vinblastine or paclitaxel for binding to tubulin has been examined and quantified by scintillation counting.	Hadfield *et al.* (2003)
Cytoblot assay	Chemical genetic approach to identify cell permeable compounds that affect the cell cycle	Chiosis *et al.* (2009)
Mass spectrometric analysis	Mass Spectrometry (MS) allowed several groups to analyze componentsof isolated MT-based structures, such as centrosomes, cilia and mitotic spindles.	Sakamoto *et al.* (2008)
Affinity column chromatography	To enrich and detect low abundant microtubule associated proteins	Sakamoto *et al.* (2008)
Fluorescent analog cytochemistry	Observation of actin filament dynamics in living cells	Small *et al.* (1999), Wang (1989)
Negative staining	The resolution of the structure of the actin filament, to the localisation of associated molecules on its surface and to the characterisation of bundled assemblies of actin filaments.	Small *et al.* (1999), Tamura *et al.* (2000)
Critical point drying	Drying is performed in a temperature-controlled pressure chamber that allows the transition from liquid to gas.	Small *et al.* (1999), Svitkina *et al.* (2007)
Quick freezing	Relies on vitreous freezing and successfully applied to tissues, suspended cells, and isolated molecules	Small *et al.* (1999), Svitkina *et al.* (2007)

based systems (e.g., liposphere, solid lipid nanoparticles, nanocore technology), colloidosomes and nanoparticles owing to their properties could be applicable for targeting the drugs and bioactives into the cytoskeleton elements (Rawat *et al.*, 2008a, b; Saraf *et al.*, 2011; Rawat *et al.*, 2006). Various agents which act into the cytoskeleton have low water solubility like paclitaxel, cytochalasin etc. and the lipid based system (liposphere, solid lipid nanoparticle, nanocore technology) (Rawat *et al.*, 2008b; Rawat and Saraf, 2008) is one of the most promising carrier for that type of agent because they provide better entrapement and stability to the low water soluble drugs (Rawat *et al.*, 2008a). Nanosuspension and nanocapsules are stable systems for controlled delivery of poorly water soluble drugs (Rad, 2010; Rawat *et al.*, 2006). Liquid crystal has been useful for the lipid soluble as well as water soluble drugs (Garg *et al.*, 2007). Nanoshells coated with gold system are very beneficial for the tumor targeting and thus it is useful for microtubule targeting. Controlled and targeted delivery is one of the most enviable requirement from a carrier which involves multidisciplinary site specific or targeted approach may be helpful to targeting the agent into actin filament microtubule and intermediate filaments.

CONCLUSION

The cytoskeleton may serve as a scaffold for the assembly of receptors and signaling molecules to realize specific intracellular signal-transduction pathways. Therefore, appropriate methods are necessary to determine conditions that induce the anchorage of receptors and intracellular signaling proteins to the cytoskeleton and different methods are helpful in determining the role of cytoskeleton in drug targeting. The conformational dynamics of cytoskeleton and the effects of different factors have been discussed. Considering the rich variety of cytoskeleton functions and the large amount of data accumulated in these studies some of the couplings between the structural changes of cytoskeleton and drug targeting were revealed. In many other cases the complete understanding of the roles of cytoskeleton for drug targeting is further studied. Recent advances in the development of novel technologies open the possibility to study the conformational dynamics of cytoskeleton in specific cellular structures associated to diverse regulatory proteins despite the different hypotheses about the exact role of cytoskeleton in drug targeting. Different analysis techniques were helpful to determine the role of cytoskeleton in drug targeting and helpful to analyze the role of cytoskeletal element in cellular and physio-pathological processes.

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