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Review Article

COLLABORATIVE ACTION OF CELL CYCLE, MOLECULAR CHAPERONES, AND UBIQUITIN PROTEASOME SYSTEM IN NEUROONCOLOGY

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Abstract: The striking feature in tumor biology is uncontrolled cell proliferation and growth. Any alteration in the genetic make up may cause cell cycle deregulation that leads to aberrant cell cycle re-entry. These cascades ultimately cause cancerous situation with unwanted cell growth and division. There are several factors in cell cycle events that can lead to cancerous situations, for instance, checkpoint breach, extracellular signals, malfunctioned protein kinases, re-expression of cyclins and cyclin-CDKs complex. A crucial function of cyclin-CDK complex is phosphorylation of retinoblastoma tumor suppressor gene that inhibits its ability to regulate the action of E2F transcription factor, which induces the gene expression and thus cause cell proliferation. To maintain the cellular homeostasis under tumorous condition, a line of protective mechanism is switched on such as availability of molecular chaperones; and if repair work fails, ubiquitin proteasome system comes in action. These regulatory mechanisms are highly conserved and play a critical role in maintaining several molecular events in the brain tumor or any stress situation. Misfolded proteins in tumor tissues are either rectified by chaperone activity upto a certain threshold or follow a degradation pathway by proteolytic activity of ubiquitin-proteasome system. In this review, we have highlighted an extensive explorative potential of molecular chaperones in combination with ubiquitin E3 ligase enzymes activities in brain tumors.

***Keywords*:** Cyclins; Heat shock proteins; Ubiquitin E3 ligase; Therapeutics

Introduction

The multipotent cells have the power to divide and generate a tumor if signalling cascades and cell cycle regulation are altered. The cell cycle is controlled by various protein kinases, which are activated by binding with their cognate cyclins. The cyclin-dependent kinases (CDKs) activity is altered during the progression of cell cycle. The cyclin is degraded by the Ubiquitin Proteasomal System (UPS) to terminate the cell division process whereas misregulated cyclin would prove detrimental for the cell cycle thereby leading to uncontrolled division or Tumor. Another class of potentially beneficial molecules are the CKI's (Cyclin dependant kinase Inhibitor; Figure 1).

Thus, the cell cycle is mediated by two types of protein modification: phosphorylation and ubiquitination. Without cyclin, CDK has little kinase activity; only the cyclin-CDK complex is an active kinase. CDKs phosphorylate their substrates on serine and threonine, so they are serine-threonine kinases and cyclins are marked with ubiquitin for proteasomal degradation.

The first molecular link between the cell cycle and cancer development was studied with respect to mutation in the p53 gene and retinoblastoma gene (Rb gene). In contrast to mutation in p53 and Rb proteins, the decreased level of CKI (p27) was also observed in most cancer patients. The clinical data shows that SKP2 (S-phase kinase-associated

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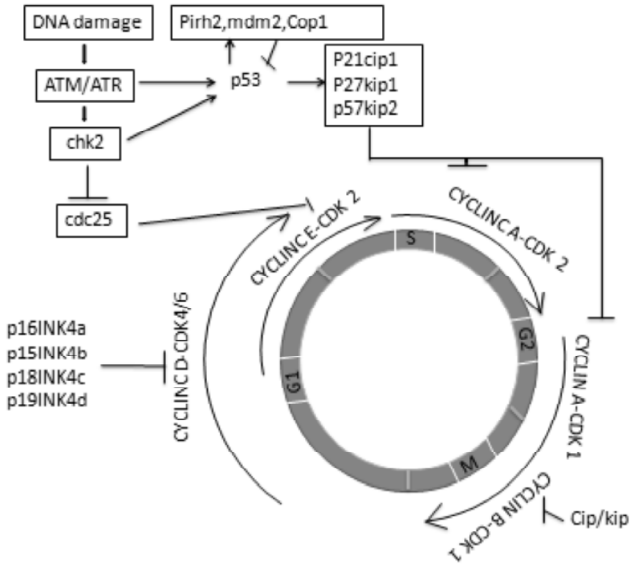

Figure 1: **Cyclin inhibitors and its function in cell cycle progression**

protein 2), an E3 ligase is overexpressed (Chen *et al.,* 2008) and marks p27 for degradation. This study revealed the association between the dysfunction of the UPS in cell-cycle control and the development of cancers.

In case of a stressed situation body takes other cytoprotective measures, like the remodelling of the non-native or mutated proteins by molecular chaperones and heat shock protein (HSP) machinery. The levels of HSP are tailored during the pharmacological or physiological insults. The HSP gene is transcriptionally activated by HSF (heat shock factor) and is switched off when the homeostasis is restored. A decade back researchers have developed a novel understanding of the use of HSP as immunological adjuvants that can help in breaking the tolerance limit of tumor antigens and causing a cytotoxic T-lymphocyte (CTL) mediated response to regress the tumor (Belli *et al.,* 2002; Manjili *et al.,* 2002).

Due to the significant recognition of HSP and E3 ligase by the immune system, their level of expression can help in the detection of cancer in early stages. Studies in the past have reflected the increased expression of Hsp27, Hsp70 and Hsp90 in various brain neoplasms (Hauser *et al.,* 2006). The Heat shock proteins and ubiquitin proteasomal system together play a crucial role in tumor cell proliferation, differentiation, invasion, metastasis, death. In this review, we provide an overview of the current status of the HSPs and E3 ligases in cancer research. The current era focuses on the development of HSP as prognostic markers for the diagnosis of cancer and also to develop anti-E3 ligase drugs for its treatment.

The cell cycle and brain cancer

The cell cycle is regulated by the dynamic interplay between the positive regulator, negative regulator, and the semi-autonomous transcriptional network that acts in consent with both. The tight regulation of cell cycle is mediated by key positive regulators cyclin along with its kinases (CDK) and negative regulators CKI's. Based on active site residue involved in the catalysis process of the CDK, they can be subdivided into Tyrosine (Tyr, Y), Serine (Ser, S)/ Threonine (Thr, T)-specific kinases. The initiation of a phase is marked by activation of protein kinases, followed up by its downregulation in order to proceed to the next phase. The downregulation of protein kinases is carried-out by negative feedback systems, which can attenuate kinase activity by the UPS system or destroy the activating regulators or can upregulate the inhibitors (CKIs). The manifestations of cancer begin with malignant phenotype due to repetitive expression of positive regulators or else loss of the negative regulators. The irregular expression levels of different cyclins along with their prognostic markers have been recorded in various neuro-oncological studies. The G1/S phase transition cyclin, CyclinD1 expression has been associated with several brain neoplasms like glioma's and meningioma (Farizan *et al.,* 2009). Its expression in brain neoplasms has been significantly correlated with markers like PCNA and MIB-1 (Alma *et al.,* 2007). Another specific D-type cyclin; CyclinD2 has showed mixed expression in gliomas and glioblastomas multiforme Denicourt *et al.,* 2008) whereas Cyclin E was associated with advanced stage of the disease and was over expressed as abnormal protein (Keyomarsi *et al.,* 1993; 1994). Few studies also reported CyclinA re-expression in correlation with an anaplastic oligodendroglioma grade (Park *et al.,* 2003). Increasing results have also reported the overexpression of CDC2/CyclinB1 in G2 phase

of gliomagenesis (Chen *et al.,* 2008). To combat this problem the body has devised mechanisms of either rectifying the aberrant proteins using the chaperones systems or tagging it with ubiquitin and guiding it towards the death path.

The role of HSP in brain tumors

Like most cancers, brain tumor cells also exhibit a highly conserved "stress response" mechanism to prevent damage. The tumor cells can be the outcome of collection of stress which may be due to ischemia, hyperthermia, hypoxia, tumor, cancer, UV radiation, heavy-metal ions, mental stress, viral infection in the cells (Sumrejkanchanakij *et al.,* 2003). In such stress situations the cells try to combat with an adverse condition by its cellular protein folding machinery. The immune-histological studies show that a stressed tumor has elevated expression of chaperone and HSPs (Table 1). The client component co-chaperone; a cognate of chaperone assists them in the remodelling of proteins. The co-chaperones and/or heat shock proteins (HSP) are cytoprotective in nature. Cells pre-exposed to these conditions develop tolerance capacity for the above kinds of stresses (Augustinack *et al.,* 2002; Hashiguchi *et al.,* 2002; Santra *et al.,* 2009) through anti-apoptotic mechanisms and helps in maintenance of proteome homeostasis.

HSPs are regulated by the activation and trimerization of transcription factor heat shock factors (HSFs) which are over expressed during tumorigenesis and plays a very crucial role in the invasion and spread of cancerous cells. The mechanism involves the stressed induced activation of HSF, which further trimerizes and bind to the upstream element HSE (Heat Shock Element) and triggers the transcription of heat shock proteins.

How does HSPs control the cell cycle machinery?

The HSP influence the regulation of the cell cycle in multiple ways. The main role of chaperone machinery is to give proper folding to a nascent protein, whose conformation has been altered due to physiological and pharmacological stress. These proteins were first identified in *Drosophila* in early seventies in response to heat (Tissieres *et al.,* 1974) and hence called the Heat Shock Proteins (HSPs).The nomenclatures of HSPs were given based on their molecular weight. The m-RNA expression results in the past have demonstrated the upregulation of mitogens, serum, and growth stimulator parallel to the HSP expression during the protein synthesis phase (Hansen *et al.,* 1991). The p53, a tumor suppressor gene, which expresses in response to a DNA damage, regulates the transition between G1/S phases. A study by Wu *et al.*, shows the regulation of p53 is highly controlled, it down regulates several

Table 1
HSP expression in brain neoplasms

Cancer type	*Involved HSPs*	*References*
Glioblsatoma	HSP90α induces ATP and Ca^{2+} release, helping in cell migration	Thuringer *et al.,* 2011
Low grade glioma	Downregulation of HSP27	Shen *et al.,* 2010
Medulloblastoma	High expression of HSP27, HSP70 and HSP90	Hauser *et al.,* 2006
Pineal parenchymal tumors	HSP27	Numoto *et al.,* 1994
Glioblastomas and anaplastic tumors	High HSP27	Hitotsumatsu *et al.,* 1996
Schwanomas and chordomas	alpha B-crystallin	Hitotsumatsu *et al.,* 1996
Glioblastoma multiforme	HSP 96	Crane *et al.,* 2012
Astrocytomas	HSP 27	Kato *et al.,* 1992
High-grade and most low-grade gliomas, including oligodendrogliomas	HSP27,HSP79,HSP90	Strik *et al.,* 2000
Primary and metastatic tumours of the brain	HSP60	Kato *et al.,* 2001

immediate early genes and Hsp70 gene promoter. While the oncoproteins stimulate Hsp70 promoters (Wu *et al.,* 1985; Milarski *et al.,* 1986) Hsp90 and other co-chaperones, they are involved in the stabilization and localization of the wild-type suppressor gene. Mutated form of p53 is associated with Hsp70, Hsp90 (Blagosklonny *et al.,* 1996) and Hsp40 (Sugito *et al.,* 1995) interaction. The HSF3/c-Myb association is necessary for Hsp expression, which is severely disrupted by binding of the p53 to HSF3, resulting in the attenuation of Hsp70 expression. The cytokines produced during the damage activate the JAK pathway. This cascade of reaction carried out by p53 allows its interaction with STAT1 and p73 and induces apoptotic signal (Townsend *et al.,* 2004). The viral oncogenesis has also established a link between DNA synthesis and HSP protein levels (Wu *et al.,* 1985; Milarski 1986). The Hsp70 has been found to be directly involved in the induction of retinoblastoma proteins. The dephosphorylated Rb binds to the EF2 transcription factor inhibits the DNA synthesis thereby not allowing the cell to progress to S phase. The Hsp70 and its co-chaperone Hsp40 have been found to stabilize the dephosphorylated Rb indeed saving the cell from undergoing repetitive divisions (Hatamoto *et al.,* 2006). Knockout studies demonstrated the decrease in level of p27 and up-regulation of Hsp70 and Hsp27 (Liu *et al.,*2010). Even under normal condition the cyclin-dependent kinase inhibitor p27 interacts with 70 kDa Hsc73 (cognate of HSP; Nakamura *et al.,* 1999). The cell cycle progression from G1/S phase till mitosis is marked by activation of CDK. The published studies have highlighted increase in the levels of chaperones in proliferating cells as well as in differentiated cells (García-Bermejo *et al.,* 1995). Another striking similarity was observed between cdk-promoter and Hsp-promoter, which signifies the fact that a relationship exist between both (Pechan *et al.,* 1995). The CDK have also been found to be dependent on HSP for their functioning and stability (Table 2).

Altogether the function of molecular chaperones ensures that the nascent polypeptide fold correctly and thereby lowering the chance of protein aggregation.

Ubiquitination proteasome system in brain tumor (UPS System)

Ubiquitination process dictates the deletion of unwanted component during the cell cycle.

Table 2
The HSP interacting with various component of cell cycle machinery

Cell cycle components	*Effect*	*References*
Cdk1	HSP90 helps Cdk-1 in substrate interaction, HSP-Cdk relation is Wee mediated	Muñoz *et al.,* 1999; Stingl *et al.,* 2010; Vassilev *et al.,* 2006; Caldas-Lopes *et al.,* 2009
Cdk2	Client of HSP90 via helix αC	Prince *et al.,* 2005
Cdk4	Cdc37 mediated interaction.	Vaughan *et al.,* 2006
Cdk6	Client of HSP90	Peng *et al.,* 2010
Myt	DAF21/Hsp90 is needed for proper folding in oogenesis	Inoue *et al.,* 2006
Wee	DAF21/Hsp90 is needed for proper Wee-1.3 stability	Inoue *et al.,* 2006
Plk-1	Metaphase -anaphase transition regulator	de Cárcer *et al.,* 2001
Aurora B	Client of HSP90 but interaction is not mandatory	Davies *et al.,* 2010
Cdk-1	Cln3-Cdk1 retention at the ER by interaction with the HSP70-related chaperones Ssa1 and Ssa2	Enserink *et al.,* 2010
Cdk2	HSP70-2 mediated modulation in mice spermatocytes	Zhu *et al.,* 1997
Cdk4	HSP90 client protein (Cdk4) inhibitor induces HSP70 accumulation. Proteosome dependent degradation	Zhang *et al.,*2008
Cdk6	HSP90 client protein(Cdk6) inhibitor induces HSP70 accumulation.	Peng *et al.,* 2010
Survivin	Level decreases in HSP60 knockdown (not cytosolic fraction)	Ghosh *et al.,* 2008

Ubiquitin is very small molecule having a molecular weight of 8kD. It is a well conserved 76 aa residue structure that binds to the target protein by a reversible mechanism (Figure 2). The ubiquitin proteasome system contains the three distinct enzymes for tagging ubiquitin to the substrate lysine residue and directing it towards the 26S proteasome that finally degrades the substrate into small peptide of 3-20 residues. Thus ubiquitin is the molecule that actually decides the fate of the substrate molecule but actual degradation is carried out by proteasome.

The ubiquitin moiety gets activated by ubiquitin activating enzyme (E1) in ATP dependent manner. The E1 activates Ub through the formation of a thiol-ester bond between the C terminus of Ub and the active site cysteine (Cys) of the E1 thereby making the c-terminal glycine residue of Ub reactive. The E1 enzyme in the second step of the process is replaced by E2 enzyme; Ubiquitin conjugating enzyme. In reactive-state Ub is transferred to the conserved cysteine residue on E2 .The final step involves the Ubiquitin E3 ligase (E3) that is a ligating enzyme with two different domains one for binding of substrate and other for the E2, which attaches Ub (from E2) to the lysine residues of the substrate protein. The process repeats itself several times thereby creating polyubiquitin chain.

Some proteins like Small Ubiquitin related MOdifier (SUMO), NEDD8, ISG15 also have been found to carry out crucial role in regulation of gene expression during tumor progression. The E3s are huge set of proteins, characterized by one or several motifs. These include a HECT (homologous to E6-associated protein C-terminus), RING (really interesting new gene) or U-box (a modified RING motif without the full complement of Zn^{2+}-binding ligands) domain. The Table 3 cites some of the ubiquitin E3 ligase that has been studied in response to various brain neoplasm.

Table 3
The response of E3 ligases in Brain cancers

Name	*Substrate*	*Function*	*Brain Cancer*	*References*
hBre1 Ubiquitin ligase	Ebp1 tumour suppressor protein (interact with E2F1)	Increase Ebp1 ubiquitation	Primary gliomas	Liu *et al.*, 2009
NARF Ubiquitin ligase	TCF/LEF transcription factors.	Could act as a negative regulator of Wnt/beta-catenine pathway or a positive regulator in colony formation in RING dependent manner.	Glioblastoma multiforme	Yamada *et al.*, 2006; Anderson *et al.*, 2010
E2C/UbcH10 Ubiquitin conjugating enzyme	Cyclin B	Probable proliferation marker. Acts with APC (E3) via N terminal extension. May induce apoptosis via p53, Bax, G2/M arrest of cell cycle.	Astrocytoma Glioma	Donato *et al.*, 2008; Jiang *et al.*, 2008; Summers *et al.*, 2008; Kobirumaki *et al.*, 2005
Huwe1 E3 ligase	Histones, Mcl1, p53, c-Myc, cdc6, N-Myc	Suppression of N-DLL3-Myc cascade	Glioblastoma multiforme	Zhao *et al.*, 2009
hDM2	p53	Poly ubiquitination of p53		Yang *et al.*, 2005
HAUSP	p53 or Mdm2	May stabilize or destabilize p53	No Report	Masuya *et al.*, 2006; Cummins *et al.*, 2004
SCF-Skp2	p27kip1	Ubiquitination of p27	Glioma	Piva *et al.*, 1999
ISG15 E3 ligase		Acts with UBP43 (Usp18) E2.Increases in cancers	Glioblastoma	Desai *et al.*, 2008

contd. table 3

Name	*Substrate*	*Function*	*Brain Cancer*	*References*
Nedd4 E3 liagse	ENaC, pTEN, IGF-1R, p63, VEGF-R2, Cbl-b, EPS15, Hrs	Blocking of Akt is majorly studied. FoxM1B mediated regulation.	Astrocytoma	Dai *et al.*, 2010
pVHL E3	HIF-1α	Recognize prolyl hydroxylase mediated proline modification on HIF-1	Hemangio blastoma	Butman *et al.*, 2008
SCF –Skp1	G_1 cyclins and p27 and p21	control G1-S progression and target G1 cyclins and CKIs for degradation	No Report	Yam *et al.*, 1999
Neuralized	degradation of Notch ligands DI	degradation of Notch ligands DI and activation of Notch signalling	Medullo blastoma	Teider *et al.*, 2010
SUMO1-3	H2AX a histone protein	Prevents DNA double-strand damage	Low-grade astrocytoma and glioblastoma multiforme	[Yang *et al.*, 2012

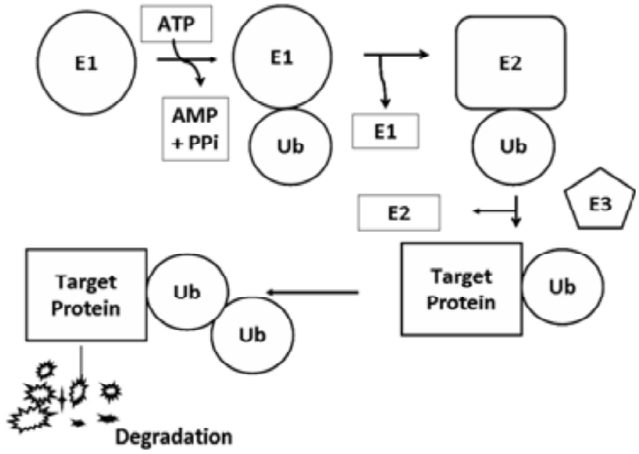

Figure 2: **The ubiquitination pathway for the degradation of non functional proteins**

Conclusion

Targeting brain neoplasms and finding a way to eliminate them has been a challeng for years now. This review provides us the possible connections and suggests the potential mechanisms to detect the onset of the disease and therapeutics based on chaperonic pathway. HSP's provides us a gateway to try and modify the altered or attenuated proteins in a cytoprotective manner where we can engineer the desire therapeutic proteins as well as a proper inhibitor for a particular site. Experiments around the world are being carried out to determine a potent inhibitor to the several important targets. Conjugation through Lys48 of the ubiquitin and the modified protein can lead to degradation through proteasomal degradation. E3 Ligases can negatively control p53 thereby decreasing its level to DNA damage and uncontrolled cell division just as it downregulates p27. Engineering of the E3 ligase can switch from a tumor promoting activity to tumor suppressing activity that helps in the regulation the expression of E3 genes in the brain neoplasm; however, inhibiting a particular ligase is still an experimental challenge. Blocking of Hdm2 with inhibitors has had other derogatory effects earlier. The best way to target the ligases should be by utilizing small molecules that could go on and specifically bind to the active site of ligases. Nanotechnology can be used to advantageous effect to design such small molecules. Further, targeting the active site of the 20s proteasome remains a viable option but at the same time there are several pertinent questions regarding the difficulties in execution such as whether these techniques can be beneficial in brain tumor cells to control the proliferation? What mechanism can be used to transport the drug to the brain neoplasm crossing the blood brain barrier? What are the potential effects of the inhibitor on the brain normal cells?

Abbreviations

CDK, cyclin dependent kinase; CIKs, Cyclin dependant kinase Inhibitor; UPS, Ubiquitin Proteasomal System; Rb, retinoblastoma gen; HSE, Heat Shock Element; HSP, heat

shock protein; HSF, heat shock transcription factor; CTL, cytotoxic T-lymphocyte; PCNA, proliferative cell nuclear antigen; E1, ubiquitin activating enzyme; E2, Ubiquitin conjugating enzyme; E3, Ubiquitin E3 ligase; SUMO, Small Ubiquitin related Modifier.

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