

## Cyclin B in Oral Squamous Cell Carcinoma: Mini Review

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### ABSTRACT

Oral squamous cell carcinoma accounts for more than 50% of all tumours in India due to the increased use of tobacco and its products. The most common cause of mortality in OSCC is due to the local recurrence and regional and distant nodal metastasis, thus necessitating the need for proper therapy. Cyclins are a family of proteins that play an important role in the cell cycle and its regulation. Cyclin B which belongs to the group of M cyclins is responsible for the G2/M transition and also the mitotic process. Cyclin B1 has been implicated in many cancers including Oral squamous cell carcinoma. Cyclin B2 which is coexpressed along with cyclin B1 is relatively less known. Cyclin B2 can act in the absence of cyclin B1 and vice versa too. Cyclin B1 can induce golgi fermentation faster than cyclin B2. Cyclin B2 can cause the progression of cell to undergo mitosis in the absence of cyclin B1. So in cancers both the cyclins play an important role. Targeted therapies have earlier targeted only the cyclin dependent kinases in the cyclin B1-Cdk complex. But now research is on to directly act against cyclin B. This mini review brings to light the role and expression of cyclin B in oral squamous cell carcinoma.

**Key words:** Cell cycle, check points, cyclin B, M phase factor, cyclin B1, cyclin B2

### INTRODUCTION

Cancer and its occurrence have become very common among both the developed and the developing countries. WHO has reported that the Oral cancer is the 8<sup>th</sup> most common cancer globally<sup>(1)</sup>. In India, Head and Neck cancer among the males is the 6<sup>th</sup> most common and among the females the 7<sup>th</sup> most common cause of death and accounts for about 1/3<sup>rd</sup> of all cancers. Oral and oropharyngeal carcinomas comprise up to half of all malignancies<sup>(2)</sup>. It presents as a lethal disease in more than 50 % of cases mainly due to the late detection resulting in patients presenting with advanced stage of cancer. OSCC presents with local invasiveness and lymph nodal metastasis and mortality is usually due to the recurrence and metastasis. Earlier Oral cancer was considered as a disease of elderly but more recently it has found to be more prevalent among young

adults due to their usage of tobacco and its products<sup>(3,4)</sup>. The role and the expression of various proteins have been studied by different authors in OSCC. In this mini review the role of Cyclin B and its expression in OSCC is explained.

### Cell cycle and its regulatory proteins

Cell cycle is a normal process which constitutes a series of events resulting in the division and duplication of a cell. There are many phases in the cell cycle. G<sub>0</sub> is the resting phase where the cell has left the cell cycle, G<sub>1</sub> is the phase where the cell enters the cell cycle and gets ready for the DNA replication, S phase is the synthesis phase where DNA replication does occur. G<sub>2</sub> is the intermediate phase where the cell continues to grow and prepares itself to enter the next phase that is the M phase or the mitotic phase where cell growth stops and the cell division occurs<sup>(5)</sup>. (Figure 1)

There are various cell cycle checkpoints that monitor and regulate the progress of the cell cycle. They assess the condition of the cell before it enters the next phase. There are 3 check points namely G1 checkpoint (restriction/starting check point), G2/M check point and the metaphase checkpoint (spindle check point)<sup>(5)</sup>. The mechanism by which the check points exert their action is by regulating the activity of the cyclin dependant kinases (CDKs) which are family of protein kinases<sup>(6)</sup>. These CDKs bind to the regulator proteins known as cyclins. The resultant complex is formed or activated at different phases and this inturn activates certain target substances which promote or prevent cell cycle progression.

Cyclins are a family of proteins that are divided into four classes based on which phase of the cell cycle they control like G1/S cyclins, S cyclins, G2 cyclins and M cyclins. Cyclin A and Cyclin B belong to the group of M cyclins which are otherwise termed as mitotic cyclins. When the cell enters the mitotic phase the concentration of M cyclins increase and reaches the peak level at metaphase. These M cyclins induces spindle formation and assembly and alignment of the sister chromatids. The destruction of the M cyclins signifies that the mitotic process is completed<sup>(7)</sup>.

### Cyclin B

It is an important protein that is required for the progression of cells from S phase to M phase and also exits the M phase. There are 3 types of cyclin B namely B1, B2 and B3. Of the three Cyclin B1 and cyclin B2 have been researched and studied<sup>(8)</sup>. Cyclin B3 is expressed in the human testis and developing germ cells in the mouse<sup>(9)</sup>. Cyclin B1 and B2 are co expressed. They are detectable beginning in G1, rise slowly through S phase then rapidly in G2, peaking in late G2 or early M, and degraded approximately after metaphase. Cyclin B requires the cdk1 for its action<sup>(10)</sup>. (Flowchart 1)

### Cyclin B1

Cyclin B1 is encoded by the CCNB1 gene. The location of cyclin b1 is in the microtubules. When the cyclin b1 localizes within the nucleus it is an indicator for cells undergoing mitotic division. In non mitotic phase the cyclin B1 is localized in the cytoplasm. This cyclin B1 coupled with the cdk1

forms the M-phase promoting factor. It is involved in the early events of mitosis such as nuclear condensation, breaking down of the nuclear envelope and spindle pole assembly. Destruction of cyclin b1 by the process of ubiquitination by the APC (Anaphase-promoting complex) leads to the exit of the cell from mitosis. Cyclin B1 accumulation is also involved in the apoptosis caused by nerve growth factor (NGF) withdrawal and T cell receptor activation. At the end of metaphase (Flow chart 2)<sup>(11,12)</sup>

The different factors which have found to increase the levels of cyclin B1 are HPV (Human papilloma virus), 17 beta- estrdiol, Insulin –like growth factor and prolactin releasing hormone which are necessary factors in normal development.<sup>(12)</sup>

### Role of Cyclin B1 in Cancer

The cyclin B1/CDK1 complex is necessary for the expression of the survival signal survivin which inturn is required for proper creation of the mitotic spindle. This brings about the polarity of the cell which is a pre requisite for proper cell division. Its levels are de regulated in case of tumours. When the levels are elevated cells enter the M phase prematurely causing a chaotic environment which is favourable for cancer. Increased numbers of cells enter the mitotic phase prematurely thereby leading to genomic instability and malignant transformation. If the levels are depleted then the cell division slows down due to the inability to form the MPF<sup>(13)</sup>. Also when cyclin B levels are disrupted then the levels of survivin also reduce which results in the loss of polarity. The dual events of reduction in survivin and mitotic disarray leads to triggering of apoptosis via the caspase 3 mediated pathway<sup>(14,15,16)</sup>. Overexpression of cyclin B1 causes suppressed expression of E-cadherin and increased expression of N-cadherin which are the transmembrane proteins that play a part in the cell adhesion and maintaining the cell structure and integrity<sup>(17)</sup>. Tumour metastasis is a process of epithelial mesenchymal transition (EMT) and spread of the tumour cells result in seeding of different locations. It has been observed that cyclin B1 induces EMT by the NF-kB dependent pathway<sup>(18,19)</sup>. (Table 1)

### Expression of Cyclin B1 in cancer

The levels of cyclin B1 can be determined through immunohistochemical analysis performed on tumour biopsies. It is a very strong indicator of prognosis in cancer and the deregulated cyclin B1 expression can be used as an indicator of the malignant potential of the tumours. Generally, elevated levels of cyclin B indicate aggressive tumour with poor prognosis<sup>(20)</sup>. In the early stages of cancer the concentration of cyclin b1 is usually high which is recognized by the immune system and antibodies are produced against the same. Measurement of the cyclin B1 antibody can be done to determine the activity of cyclin B1 and the tumour.<sup>(21)</sup> The localization of cyclin B1 also serves as a good indicator of the aggressiveness of the tumour. When the cell is in mitotic phase cyclin b1 is present in the nucleus and in the non mitotic phase it is present in the cytoplasm<sup>(13)</sup>. Overexpression has been observed in many cancers such as prostate cancer<sup>(17)</sup>, oesophageal squamous cell carcinoma<sup>(18,20)</sup>, breast cancer<sup>(19)</sup>, tongue<sup>(22)</sup>, OSCC<sup>(23)</sup>, lung cancer<sup>(23)</sup>, etc. Nozoe *et al.*, 2002 observed that the nuclear expression of cyclin B1 showed better prediction of prognosis in esophageal SCC than the cytoplasmic expression<sup>(24)</sup>. Patil *et al.*, 2013 observed that the cyclin B1 tends to move from the cytoplasm to the nucleus with grades of OSCC thus increasing the mitotic index in higher grades<sup>(25)</sup>. Watanabe *et al.*, 2010 reported that cyclin B1 is exported early to the nucleus by altering the nuclear import and export balance in cases of OSCC which correlated with tumour differentiation<sup>(26)</sup>. De Spindula *et al.*, 2011 have found that the expression of the cell cycle proteins like cyclin B1 correlate with increase in Ki67 levels which reflects the proliferation states and thereby regulate carcinogenesis<sup>(27,28)</sup>.

### Cyclin B2

Cyclin B2 is located in the golgi region and is encoded by the gene CCNB2. The function of cyclin B2 is to tune the mitotic timing through the regulation of a golgi check point. It is found to play a role in regulating golgi fragmentation. Cyclin B2 co-localizes with Golgi markers during interphase but is cytoplasmic after Golgi fragmentation, and disperses throughout the cells after Nuclear envelop breakdown. A small amount could be shown to localize with the spindle at metaphase<sup>(29,30)</sup>. It binds

to TGF beta and thereby exerts TGF beta mediated cell cycle control. It has a direct inhibitory effect on the G2 checkpoint kinases<sup>(31)</sup>. Depletion of cyclin B2 results in reduction in the fraction of mitotic cells thereby suggesting that cyclin B2 performs a rate limiting function in the cell cycle. Depletion results in longer G2 phase but reduced M phase<sup>(32,33)</sup>.

### Expression of cyclin B2 in cancer

Takashima *et al.*, 2013 have found that strong expression of cyclin B2 mRNA correlated with poor prognosis in patients with non-small cell lung cancer<sup>(34)</sup>. Cyclin B2 transcription is shut down by expression of wild type p53<sup>(35)</sup>. Deletion of the DNA methyl transferase gene (DNMT1) leads to increased expression of cyclin B2 by increasing its transcription.<sup>(36)</sup> Cyclin B2 causes aurora-A-mediated Plk1 hyperactivation, resulting in accelerated centrosome separation and lagging chromosomes. Studies have reported that cyclin B2 and p53 act antagonistically to control aurora-A-mediated centrosome splitting and accurate chromosome segregation in normal cells. This suggests that there is a link between cyclin B2 and p53 in tumour pathophysiology<sup>(35)</sup>. Cyclin B2 has been studied in tumors such as breast cancer<sup>(37)</sup>, colorectal cancer<sup>(38)</sup>, adenocarcinoma<sup>(38)</sup> and prostate cancer<sup>(38)</sup>. It has not studied in tumours such as Oral squamous cell carcinoma.

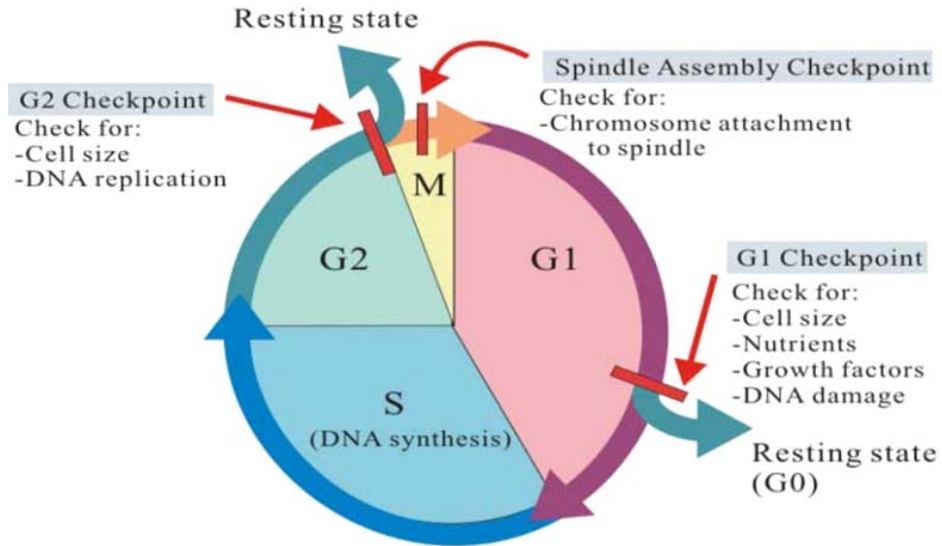
### Treatment

Most cancer targeted therapies have targeted the cdk1 subunit but there is an increased scope and research has been initiated to target the cyclin B levels in tumours. Resistance to therapy has been observed in cases where there is over expression of cyclin B1 and therapy was targeted at the cdk1 subunit. By decreasing the cyclin B levels in cells it was observed that there was an increase in the levels of functional p53. Effective treatment against cancers can be initiated where p53 function is inhibited but the gene has not been deleted. Lowering of cyclin B levels restores the tumor suppressing function of p53 and also prevents cancer cells from dividing as a consequence of low cyclin B<sup>(35)</sup>. Reversine<sup>(39,40,41)</sup> a relatively newer broad spectrum antitumour agent was found to retard the cell cycle at the G2/M stage where studies have shown that there was a prolonged expression of cyclin B1 causing malignant transformation. N-

Benzoyl- staurosporine (PKC412) <sup>(39,40,41)</sup> has found to target the cyclin b1-cdk complex. Bortezomib<sup>(42)</sup> is another proteasome inhibitor which was found to act against tumours by the cyclin B1 pathway thereby bringing G2/M cell cycle arrest.

**CONCLUSION**

Cyclin B which are the mitotic cyclins have found to play a role in many hallmarks of cancer such as hyperplasia, tumour metastasis etc. Of the three cyclins cyclin B1 and B2 have been studied. Cyclin B1 have found to play a major part in



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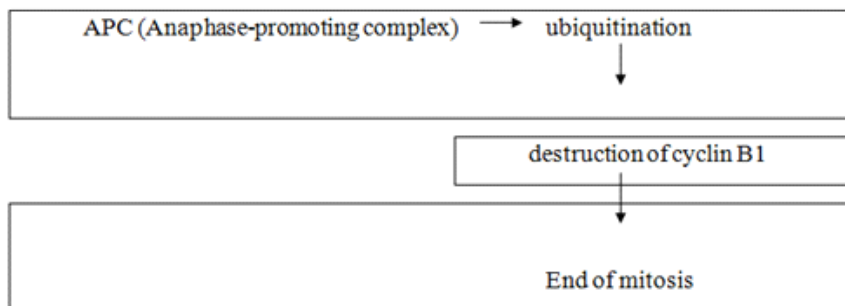
**Fig. 1: Cell Cycle and Checkpoints**

Cyclin B +cdk1 =Maturation promoting factor or M-phase promoting factor

↓ phosphorylation

activation of other proteins → mitotic events

**Flowchart 1: Cyclin B in mitosis**



**Flowchart 2 : End point of Mitosis**

**Table 1: Cyclin B1 in Cancer – Pathways involved**

Over expression of Cyclin B1	Cells enter cell cycle prematurely and overriding of the G2-M checkpoint leading to genomic instability Suppression of E-Cadherin and increased expression of N-Cadherin leading to alteration in cellular adhesion Downregulates p53 and upregulates c-Myc oncogene causing increased proliferation Induction of Epithelial Mesenchymal transition through NF-kB dependent pathway	Cancer
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tumourigenesis. Cyclin B2 though not very active but still found to be playing its part. In the absence of cyclin B1, cyclin B2 have found to aid the cell to progress from G2 phase to mitosis, even though they are co expressed. Alteration in the transition rates and the timing of mitosis has been observed in the absence of cyclin B1 / cyclin B2. Cancer targeted therapies have been directed mainly towards the cyclin dependent kinases but there has been a growing interest towards the cyclins especially cyclin B1. Studies have been performed

and clinical trials have been done to bring about a drug to act against cyclin B1. Multidrug therapy which is the treatment followed in the present day includes drugs which brings about cell cycle arrest. This review brings to light the fact that cyclin B2 can still make the cell to progress in the cell cycle independent of cyclin B1 and also playing a role in tumourigenesis via separate pathways. Thus studies are warranted to document the role of cyclin B2 in cancers especially OSCC and also develop strategies and therapies against the same.

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