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**The Role of Cyclin D1 Gene in Head and Neck Carcinoma**

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**ABSTRACT**

Cyclin D1 acts as an oncogene in different humanneoplasia when over expressed. To date, no CCND1 mutations have been reported, but cyclin D1 over expression may be the result of CCND1 rearrangement or amplification. CCND1 amplifications have been reported in several human neoplasia, such as in situ and infiltrating ductal breast carcinoma, and in bladder, head and neck, lung and prostate cancer.Scientists demonstrated that cyclin D1 turnover is governed by ubiquitination and proteasomal degradation, which are positively regulated by cyclin D1 phosphorylation of threonine 286. Although CDK4- bound cyclin D1 molecules are intrinsically unstable (half-life ~30 min), a cyclin D1 mutant (T286A) containing an alanine for threonine 286 substitution failed to be efficiently polyubiquitinated, and was markedly stabilized (half-life approximately 3.5 h) when inducibly expressed. These authors also demonstrated that GSK-3b phosphorylates cyclin D1 specifically at threonine 286, thereby triggering rapid cyclin D1 turnover, while the cyclin D1 mutant T286A was refractory to phosphorylation by GSK-3 and remained in the nucleus throughout the cell cycle. This study provides a compilation of previously reported mechanism of cyclin D1 over expression in human tumors in vivo.

Keywords: Cyclin D1,Carcinoma,Cell Cycle,Oncogene,Neoplasia

1. **INTRODUCTION**
	1. **Cancer**

Cancer is a group of many related diseases that begin in cells, the body's basic unit of life. Normally, cells grow and divide to form new cells in an orderly way. They perform their functions for a while, and then they die. Sometimes, however, cells do not die. Instead, they continue to divide and create new cells that the body does not need. The extra cells form a mass of tissue, called a growth or tumor. There are two types of tumors: benign and malignant. Benign tumors are not cancer. They do not invade nearby tissue or spread to other parts of the body. Malignant tumors are cancer. Their growth invades normal structures near the tumor and spreads to other parts of the body. Metastasis is the spread of cancer beyond one location in the body.

* 1. **Head and Neck Cancer**

The term head and neck cancer refers to a group of biologically similar cancers originating from the upper aerodigestive tract, including the lip, oral cavity (mouth), nasal cavity, paranasal sinuses, pharynx, and larynx. Most head and neck cancers are squamous cell carcinomas, originating from the mucosal lining (epithelium) of these regions [1]. Head and neck cancers often spread to the lymph nodes of the neck, and this is often the first (and sometimes only) manifestation of the disease at the time of diagnosis. Head and neck cancer is strongly associated with certain environmental and lifestyle risk factors, including tobacco smoking, alcohol consumption, and certain strains of the sexually transmitted human papillomavirus. Head and neck cancer is highly curable if detected early, usually with some form of surgery although chemotherapy and radiation therapy may also play an important role.

In the past two decades, several authors have detected HPV DNA in head and neck cancers by Southern blot hybridisation (SBH) or polymerase chain reaction (PCR) [1]. Among head and neck cancers, HPV DNA positivity tends to show site dependence, with the tonsils, oral cavity, and larynx being the most common sites [2] High risk HPV types 16 and 18 are by far the most predominant types at all sites [3,4]. However, epidemiological data reveal that the role of HPVs in the aetiology of head and neck cancers is rather controversial: the reported frequency of HPV DNA even in the often studied laryngeal site varies between 3% and 85% in the literature. In addition, the prognostic value of HPV DNA positivity is also equivocal [5].

HPV DNA may exist either in the episomal form or integrated into the host’s genome [6]. In the episomal form, the circular double stranded papillomavirus DNA is intact. In contrast, integration results in disruption of the circular viral genome, mainly in the E1E2 open reading frames (ORFs) [7].The product of the E2 ORF has a regulatory role in the transcription of the transforming E6 and E7 viral genes.

Disruption of this regulatory role at integration results in increased concentrations of transforming viral proteins E6 and E7, which interact with the p53 and retinoblastoma tumour suppressor proteins, respectively, and therefore alter cell cycle control [7].The physical state of HPV DNA tends to correlate with histopathological findings in the uterine cervix. Viral DNA is present as an episome in low grade cervical intraepithelial lesions, whereas the integrated form is predominant in high grade cervical intraepithelial lesions and invasive tumours, although integration is not an essential prerequisite for malignant progression [8]. Thus, the prognostic role of integration in cervical cancer remains unclear [9].

* 1. **CYCLIN D1**

The cyclin D1 gene (CCND1) encodes cyclin D1 protein, which is expressed in response to mitogenic signals promoting transition through the restriction point in the G1 phase of the cell cycle [10]. Increased expression of cyclin D1 has been associated with increased cell proliferation [11]. CCND1 amplification leading to deregulated CCND1 expression is common in tumors from patients with SCCHN [12]. Cyclin D1 protein over expression has been shown to correlate with reduced 5-year and overall survival in SCCHN patients [13]. Other studies have shown that cyclin D1 protein over expression is associated it with poor prognosis in primary hypopharyngeal, laryngeal, esophageal, and oral squamous cell carcinomas [14]. Furthermore cyclin D1 antisense experiments have demonstrated that the gene may be a potential target for

Therapeutic intervention in SCCHN [15]

**2.1. Cyclin D1:**

Cyclin D1 belongs to the family of cyclin proteins which functions as the regulatory subunits of cyclin/cyclin dependent kinase (cdk) holozymes that regulate the entry into the progression through the cell cycle.

Of the three D-type cyclins, cyclin D1 remains the most extensively studied, largely because of its frequent over expression in human malignancy. In addition to transcriptional and translational regulation, cyclin D1 is subject to growth factor dependent post-translational regulation. G1 phase serves as the point wherein growth factor signaling integrates with the cell cycle and cell division. Growth factor signaling through Ras-dependent pathways increases cyclin D transcription, translation and ultimately promotes binding to either CDK4 or CDK6 [16]. Activation of the assembled cyclin D1/ CDK4 complex requires phosphorylation by the CDK-activating kinase (CAK) in turn

**2.2.** **Importance of Cyclin D1 complex:**

Cyclin D1/CDK4 activity is kept in check by several distinct regulatory events. Proteolysis of cyclin D1 requires poly-ubiquitination, which targets cyclin D1 to the 26S proteasome [17]. Poly-ubiquitination of cyclin D1 requires phosphorylation of a conserved C-terminal threonine, Thr-286, by the glycogen synthase kinase 3 beta, GSK-3b. GSK-3b is excluded from the nucleus during G1 phase, but enters the nucleus upon S-phase entry thereby gaining access to the nuclear cyclin D1-CDK4 complex [18].Phosphorylation of cyclin D1 on Thr-286 then triggers a coupled event. Phosphorylated Thr-286 is first bound by the CRM1 nuclear exportin, which then shuttles the cyclin D1 complex to the cytoplasm. Once in the cytoplasm, phospho-cyclin D1 is then targeted by an as yet unidentified E3 ubiquitin ligase, ubiquitinated and thereby marked for destruction. In addition to regulation via nuclear export coupled proteolysis, cyclin D-CDK kinase activity is opposed by direct binding with small polypeptide inhibitors of which p16INK4a is the prototypical member [19].

The control of cyclin D1 degradation by ubiquitination in the 26S proteasome is important for maintaining appropriate cyclin D1 levels during the cell cycle. Phosphorylation of cyclin D1 at threonine 286 is required for its ubiquitination, nuclear export and degradation in the cytoplasm [20,21, 22, 23,]. This phosphorylation is mediated by glycogen synthase kinase 3- β (GSK3- β ) and is greatly enhanced by the binding of cyclin D1 to CDK4.

The binding of p21CIP1 and p27KIP1 with cyclin D1 and CDK4 serves a dual function. The first is that p21CIP1 and p27KIP1 facilitate stable association of the cyclin D-CDK4 complex[24] . Indeed cells lacking both p21CIP1 and p27KIP1 are unable to form active D1/CDK4 complexes [24,25,26]. Second, the stable association of p21CIP1 and p27KIP1 with cyclin D1-CDK4 relieves CDK2 from the CIP/KIP inhibitory activities thereby indirectly promoting CDK2 activity. Therefore, induction of cyclin D1 during G1 drives Rb phosphorylation and promotes CDK2 activity by titrating the CDK2 inhibitory proteins p21CIP1/p27KIP1 [27].

**2.3. Regulation of Cyclin D1 Degradation:**

Cyclin D1 is localized predominantly in the nucleus of asynchronously growing cells [28]. During cell cycle progression, protein levels of the cyclin begin to rise early in G1, prior to its rapid nuclear export and degradation within the cytoplasm. The nuclear export of cyclin D1 has been shown to require prior phosphorylation on Thr-286 by glycogen synthase kinase 3β (GSK3β) [29]. This phosphorylation of cyclin D1 was initially thought to regulate its ubiquitin-dependent degradation. Indeed, mutation of Thr-286 to alanine resulted in increased stability of the cyclin. Nevertheless, it is still generally believed that cyclin D1 accumulates within the nucleus during G1, and at the G1-S-phase transition, GSK3β accumulates in the nucleus and mediates phosphorylation, nuclear export and subsequent ubiquitindependent degradation of cyclin D1 in the cytoplasm [29].

The association of cdk4 with D-type cyclins to form functional kinase complexes is comparatively inefficient. This has led to the suggestion that assembly might be a regulated step. In this report we demonstrate that the CDK inhibitors p2lCIP, p27KIP, and p57KIP2 all promote the association of cdk4 with the D-type cyclins. This effect is specific and does not occur with other cdk inhibitors or cdk-binding proteins. Both in vivo and in vitro, the abundance of assembled cdk4/cyclin D complex increases directly with increasing inhibitor levels. The promotion of assembly is not attributable to a simple cell cycle block and requires the function of both the cdk and cyclin-binding domains. Kinetic studies demonstrate that p21 and p27 lead to a 35- and 80-fold increase in Ka, respectively, mostly because of a decrease in Koff,. At low concentrations, p21 promotes the assembly of active kinase complexes, whereas at higher concentrations, it inhibits activity. Moreover, immunodepletion experiments demonstrate that most of the active cdk4-associated kinase activity also associates with p21 [24].

**3. CYCLIN D1 AND CANCER**

Cyclin D1 acts as an oncogene in different human neoplasias when over expressed [30]. To date, no CCND1 mutations have been reported, but cyclin D1 over expression may be the result of CCND1 rearrangement or amplification. CCND1 amplifications have been reported in several human neoplasias, such as in situ and infiltrating ductal breast carcinoma, and in bladder, head and neck, lung and prostate cancer [29,31] demonstrated that cyclin D1 turnover is governed by ubiquitination and proteasomal degradation, which are positively regulated by cyclin D1 phosphorylation of threonine 286. Although CDK4- bound cyclin D1 molecules are intrinsically unstable (half-life ~30 min), a cyclin D1 mutant (T286A) containing an alanine for threonine 286 substitution failed to be efficiently polyubiquitinated, and was markedly stabilized (half-life approximately 3.5 h) when inducibly expressed [31]. These authors also demonstrated that GSK-3b phosphorylates cyclin D1 specifically at threonine 286, thereby triggering rapid cyclin D1 turnover, while the cyclin D1 mutant T286A was refractory to phosphorylation by GSK-3 and remained in the nucleus throughout the cell cycle (Diehl et al., 1998) this study provides the first evidence of a previously unreported mechanism of cyclin D1 over expression in human tumors in vivo: CCND1 mutations affecting critical residues involved in protein degradation [32].

Genetic aberrations in the regulatory circuits that govern transit through the G1 phase of the cell cycle occur frequently in human cancer, and over expression of cyclin D1 is one of the most commonly observed alterations Cyclin D1 is amplified and/or over expressed in a substantial proportion of different human tumors. Increased cyclin D1 abundance occurs relatively early during tumorigenesis [30]. Moreover, deregulation of cyclin D1 either by overexpression or mutation may contribute to the resistance of head and neck squamous cell carcinomas to EGFR inhibitors and other known drugs for cancer, thus the role of cyclin D1 as a prognostic marker warrants additional analysis. Cyclin-dependent kinases (CDKs) are critical regulators of cell cycle progression and RNA transcription [34].

Increased levels of cyclin D1 occur in a large segment of human cancers. Amplification of the cyclin D1 locus accounts for a low percentage of the total number of cancers that over express cyclin D1; the mechanisms contributing to cyclin D1 over expression in the remainder has not firmly been established. Strikingly, a large body of work strongly suggests that enforced overexpression of cyclin D1 is not likely to be the essential transforming property of cyclin D1. More recently, work has revealed that expression of a cyclin D1 mutant that is refractory to nuclear export and proteolytic degradation at the G1/S boundary is a highly transforming mutant and functions independent of additional oncogenes in vitro. This finding reveals a previously unappreciated role for regulated nuclear export in harnessing cyclin D1-CDK4 activity and suggests that retention of this kinase in the nucleus during S-phase is a cancer promoting or predisposing event.

**3.1. Phosphorylation-Dependent Cyclin D1 Nuclear Export in Cancer**

Initial work identified the GSK-3b kinase as the protein kinase that phosphorylates cyclin D1 at Thr-286 thereby targeting cyclin D1 for nuclear export and proteolysis [18]. Further observations revealed that this a novel cyclin D1 isoform, cyclin D1b, whose expression results from alternative splicing[35]. Unlike the canonical cyclin D1a transcript, D1b lacks the fifth exon containing both the GSK-3b phosphorylation site and the Crm1 binding site; consequently cyclin D1b is constitutively nuclear (Lu et al., 2003). Cyclin D1b still binds and activates CDK4 and is able to disassociate an Rb/MCM7 complex demonstrating the protein product of the alternative transcript is capable of executing the known functions of the D1/CDK4 holoenzyme. The D1b protein was expressed in several tumor cell lines and was also detected in primary esophageal carcinoma tissue. Expression of cyclin D1b was not detectable in non-malignant tissue demonstrating that the D1b protein expression is cancer specific [35]. In addition, immunohistochemical staining for D1b revealed its expression and predominately nuclear localization in primary tumor tissue providing further evidence that alterations promoting cyclin D1 nuclear localization are present in human cancers.

Finally the machinery the cell utilizes to ubiquitinate phosphorylated cyclin D1 may also be inactivated in tumor cells. Given that cyclin D1 ubiquitination is coupled with cyclin nuclear export, it is conceivable that inactivation of cyclin D1 ubiquitination might also impact on its nuclear accumulation. Currently the E3 ubiquitin ligase that specifically recognizes cyclin D1 is not identified. However, by analogy with the cyclinE ubiquitin ligase Fbw7, which is directly targeted in human cancer [36,37], it is reasonable to expect that the cyclin D1 ubiquitin ligase may also be targeted during cancer genesis.

**4. VARIOUS OTHER FUNCTIONS OF CYCLIN D1 GENE**

1. Regulation of Nuclear Hormone Receptor

2. Regulation of Adipogenic Transcription Factors and Adipogenesis

3. Repression of STAT3 by Cyclin D1

4. Regulation of B-Myb Activity by Cyclin D1

5. Association with HATs, HDACs, and chromatin remodeling proteins.

**Conclusion**

The data from the various studies shows that *CCND1* polymorphism may have some protective effect against the risk of head and neck squamous cell carcinoma but for reaching any solid conclusion further studies are required.

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