CDC73, also known as *HRPT2*, is a tumour suppressor gene whose expression is lost or downregulated in parathyroid, renal, breast, uterine and gastric cancers. However, the reports regarding the role of CDC73 in oral squamous cell carcinoma (OSCC) are lacking. As part of the Paf1 complex, it remains associated with ribonucleic acid (RNA) polymerase II and is involved in transcript site selection, transcriptional elongation, histone H2B ubiquitination, histone H3 methylation, poly(A) length control and, coupling of transcriptional and posttranscriptional events. It has been reported to negatively regulate cellularproliferation by targeting oncogenes *CCND1* (*cyclin D1*) and*MYC* (*c-Myc*). Moreover, it has also been indicated to inhibitβ-catenin-mediated transcription. Taken together, these findings strongly suggest that it contributes to the expression of genes whose products have an important role in the suppression of tumor development and cell death. In this study, we have attempted to study the transcriptional and posttranscriptional regulation of *CDC73* and its role in OSCC.

The main findings of the present study are listed below.

1. To begin with, the expression analysis of *CDC73* was performed both at the RNA and the protein levels by qRT-PCR and IHC, respectively. As expected, a majority of the OSCC samples showed downregulation of CDC73 both at the RNA and the protein levels compared to their normal oral tissues.

2. Loss-of-heterozygosity (LOH), mutation and promoter methylation are the hallmarks of a tumor suppressor gene (TSG). Therefore, to characterize *CDC73* as a TSG in OSCC and to look into the mechanisms that could be the cause of CDC73 downregulation in OSCC, LOH, mutation and promoter methylation of *CDC73* were studied. The results showed that LOH, mutation and promoter methylation are not the major causes of CDC73 downregulation in OSCC.

3. To identify the alternate mechanisms as the cause of CDC73 downregulation in OSCC, a combination of bioinformatics and molecular approaches were used. The results showed that the upregulation of an inhibitory transcription factor WT1 (Wilms tumor protein 1) and an oncogenic microRNA-155 are the major causes of its downregulation in OSCC.

4. The luciferase reporter assay of SCC131 cells co-transfected with a *WT1* construct and a *CDC73* promoter construct showed that WT1 overexpression represses *CDC73* expression in a dose-dependent manner.

5. Due to the presence of zinc fingers in its C-terminal half, WT1 has been found to be a potent transcriptional regulator of genes. Therefore, to determine if WT1 downregulates *CDC73* via binding its promoter, the chromatin immunoprecipitation (ChIP) assay was performed. The results showed the binding of WT1 to the *CDC73* promoter *in vivo*. Binding of WT1 to the *CDC73* promoter was further confirmed *in vitro* by the electrophoretic mobility shift assay (EMSA).

6. The 5-aza-2'-deoxycytidine (AZA) treatment of SCC131 cells led to upregulation of *WT1* with a concomitant downregulation of *CDC73*. The COBRA technique demonstrated that the upregulation of *WT1* upon the 5-AZA treatment was due to its promoter methylation.

7. To determine if the WT1-mediated reduction of *CDC73* expression has a functional relevance in cell growth and proliferation, we knocked down CDC73 expression by transient overexpression of WT1 in SCC131 cells and quantitated cell proliferation by the MTT assay. As expected, the results demonstrated that the reduced CDC73 level was associated with an increased cell proliferation. Cotransfection of *CDC73* with *WT1* in SCC131 cells attenuated the pro-oncogenic effect of WT1 by apoptosis induction.

8. After validating *CDC73* as the target of WT1 by bioinformatics and *in vitro* assays, we quantitated the expression levels of *WT1* and *CDC73* by qRT-PCR in OSCC samples and their matched normal oral tissue samples. The results showed an inverse correlation between the expression levels of *WT1* and *CDC73* in a majority of the samples. To exclude the possibility of alternate mechanisms as the cause of *CDC73* downregulation in OSCC, we selected a subset of OSCC samples with downregulated level of *CDC73* and analysed them for LOH at the CDC73 locus and promoter methylation. Further, some of these OSCC samples were also analyzed for mutations in *CDC73*. The results showed that these OSCC samples did not have LOH, promoter methylation or any mutation, again validating the fact that *CDC73* is a biological target of oncogenic WT1, and the transcriptional repression of *CDC73* by WT1 could be a major mechanism for *CDC73* downregulation in OSCC.

9. Recent studies have shown that a growing class of noncoding RNAs called microRNAs (miRNAs) is involved in posttranscriptional regulation of genes. There is a growing body of literature supporting the potential role of miRNAs in tumorigenesis. The importance of *CDC73* in orchestration of several cellular functions and its role in tumorigenesis make it an attractive candidate for miRNA-mediated regulation of cell growth and proliferation. Using bioinformatics approaches, we identified an oncogenic microRNA-155 (miR-155) that could posttranscriptionally regulate CDC73 expression.

10. Consistent with its oncogenic role, miR-155 was found dramatically upregulated in OSCC samples and was found to be another mechanism for downregulation of CDC73 in a panel of human cell lines and a subset of OSCC samples in the absence of LOH, mutations and promoter methylation.

11. miRNAs regulate posttranscriptional gene expression generally via binding to their cognate sites in the 3'UTR. Therefore, a luciferase reporter construct was made by cloning the 3'UTR of *CDC73* downstream to the luciferase reporter gene and the reporter assay was performed. Our experiments clearly indicated that the mature miR-155 regulates *CDC73* expression by interacting with its 3'UTR in a site specific manner.

12. Ectopic expression of miR-155 in HEK293 cells dramatically reduced CDC73 levels, enhanced cell viability and decreased apoptosis. Conversely, the delivery of a miR-155 antagonist (antagomir-155) to KB cells overexpressing miR-155 resulted in increased CDC73 level, decreased cell viability, increased apoptosis and marked regression of xenografts in nude mice. Cotransfection of miR-155 with *CDC73* in HEK293 cells abrogated its pro-oncogenic effect. Reduced cell proliferation and increased apoptosis of KB cells were dependent on the presence or absence of the 3'UTR in *CDC73*.

In nutshell, the knockdown of CDC73 expression due to overexpression of WT1 and miR-155 not only adds a novelty to the list of mechanisms responsible for its downregulation in different tumors, but the restoration of CDC73 levels by the use of inhibitors to WT1 and antagomir-155 may also have an important role in therapeutic intervention of cancers, including OSCC.