RESEARCH HIGHLIGHT

# Combinatorial Transcription Regulation of Oncogene *CDC6* in Androgen-Sensitive Prostate Cancer Cells

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**Previous studies showed that *CDC6* gene transcription mainly is regulated by E2F transcription factors. In a paper we recently published [1], we reported a novel regulatory mechanism of aberrant *CDC6* gene transcription in androgen-responsive prostate cancer (PCa) cells. FOXM1 transcription factor positively regulates *CDC6* gene transcription and DNA replication. In addition to the direct binding to CDC6 promoter, FOXM1 regulates *CDC6* gene transcription by elevating AR gene expression. Furthermore, FOXM1 and AR collaboratively regulate *CDC6* gene transcription in a cell cycle-dependent manner. Based on this mechanism, siomycin A, a proteasome inhibitor known to inhibit FOXM1 expression and activity, inhibited PCa cell proliferation and its effect was additive to that of bicalutamide, an antiandrogen commonly used to treat PCa patients.**

***Keywords:*** Gene Transcription; Transcription factors; FOXM1; AR; CDC6; Prostate cancer

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Gene expression is a complicated process involving numerous steps such as transcription, translation and subsequent protein production. The gene expression aberrance of oncogenes and tumor suppressor genes contribute to tumor transformation, progression, invasion and metastasis. Gene expression and its regulation are mostly dependent on the levels of gene transcription.

Gene transcription is controlled by a complex molecular network containing genetic and epigenetic pathways. In genetic pathways, transcription factors, by forming complexes with other transcription factors, chromatin modifiers, and cofactor proteins, bind together and assemble upon the *cis*-regulatory DNA elements in the regions of promoter to regulate gene transcription [2]. In addition, epigenetic-associated molecules, including DNA methyltransferases (DNMTs), Small non-coding RNAs, long non-coding RNAs regulate gene transcription through DNA methylation and histone modification mechanisms [3].

Recent high-throughput whole genomic analysis has generated a large amount of data identifying the over or down-regulated genes in the specific cancer types and the stages of cancer progression [4, 5]. The oncogenes,

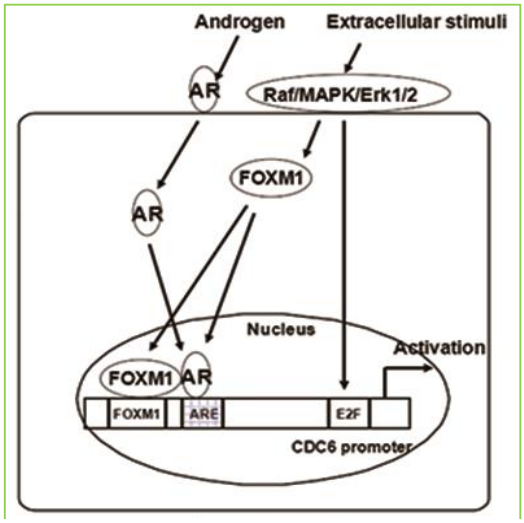


**Figure 1. Schematic Diagram showing the model of FOXM1 and AR coordinately regulate the CDC6 gene transcription.**

together with the associated oncogenic transcription factors, are significantly over-expressed in the malignant tissues comparing to the non-malignant tissues [1]. However, the mechanisms by which the tumor/tissue- specific oncogenes or tumor suppressor genes are turned on/off at the right time are still elusive, and it is most unknown how multiple oncogenic transcription factors, when highly expressed in the same cancer cells, work together to determine the specificity of gene transcription.

Cell division cycle 6 (CDC6), as an essential regulator of DNA replication, helps load a pre-replication complex to the origins of DNA replication in early G1 phase, and initiates DNA replication during S phase. Additionally, CDC6 coordinates S phase and mitotic entry, and as a license factor, ensures that the genome is replicated only once during each cell division cycle [6]. The deregulation of CDC6 results in aberrant DNA replication, DNA damage and genomic instability, and even contributes to the tumorigenesis [7]. Aberrant CDC6 expression has been reported in several cancer types such as brain cancer, non-small cell lung carcinomas, lymphomas and prostate cancer (PCa) [6, 8].

Previous studies showed that *CDC6* gene transcription is regulated by E2F transcription factors [9]. In the paper we recently published [1], FOXM1 and AR transcription factors were found to coordinate *CDC6* gene transcription in addition to E2F pathways. The growth of androgen-sensitive PCa cells depends on androgen activating AR [10]. It has been reported that AR regulates *CDC6* gene transcription and DNA replication. AR protein degraded during mitosis to re-initiate DNA replication in the next cell cycle, and is considered as one of the licensing factors contributing to the initiation of DNA replication [11]. AR, as a component of pre-replication complex, drives G1 to S phase progression of LNCaP cells through protein interaction with CDC6 [12]. In PCa cells, AR regulates CDC6 gene expression in a cell cycle dependent manner, and the ARE binding motif at the CDC6 promoter is responsible for androgen-dependent *CDC6* transcription [13]. In addition to AR effect alone, androgen regulates the gene transcription of *CDC6* through interactions between AR and E2F1 and E2F3 transcription factors [14].

In addition to AR binding elements (ARE), a forkhead box (FOX) *cis*-regulatory binding motif 5'-TGTTTGTT-3' has been identified at the CDC6 proximal promoter [15], and the motif is occupied by FOXM1 protein. The FOX binding motifs are sufficient to drive FOXM1-activated *CDC6* gene transcription. Similar to AR, FOXM1 expression is cell cycle specific, and FOXM1 protein degraded during the mitotic exit [7]. Recent evidence suggests that FOXM1 may be involved in DNA replication, and that silencing of FOXM1 blocked the cell cycle transition from G1 to S phase [16]. The similarity of function supports a possible functional and structural interaction between AR and FOXM1 proteins in the gene transcription of *CDC6*.

Interestingly, in addition to the direct DNA binding, FOXM1 regulates *CDC6* gene transcription by positively regulating AR gene expression, which suggested a novel regulatory mechanism of *CDC6* gene indirectly controlled by FOXM1. Androgen-activated AR or cell stress-activated FOXM1 such as Raf/MAPK/ERK1/2 [15] have been involved in the gene transcription regulation of CDC6 via nuclear translocation. FOXM1 and AR proteins coordinated the *CDC6* gene transcription level, highly expressed at S phase during the cell cycle. The binding of FOXM1 and AR proteins to the CDC6 promoter is relatively high at S phase. The protein levels of AR or FOXM1 influence their mutual binding to the CDC6 promoter. Androgen increased AR binding. Decline of either AR or FOXM1 protein levels resulted in the decrease of AR binding to CDC6 promoter while the decreased AR did not change FOXM1 binding to *CDC6* promoter (Fig.1). Based on these aforementioned facts, we deduced that the mechanisms by which FOXM1 protein influenced the DNA binding of AR protein to CDC6 promoter might result from two possible pathways. First, the knockdown of FOXM1 decreased AR protein levels

because FOXM1 regulated *AR* gene transcription. Additionally, FOXM1, similar to FOXA1, might play a novel role as a pioneer factor for AR protein binding to *CDC6* promoter and regulate the gene transcription of *CDC6* and other androgen-activated genes [17, 18].

Certainly, our study only revealed one part of gene transcription regulation of genetic and epigenetic molecular network of *CDC6* gene in androgen-sensitive PCa cells, and focused on FOXM1 and AR when they are highly expressed with high activity in the same cancer cells at the same time. Further studies are required to clarify the exact roles of FOXM1 as a pioneer factor in the epigenetic regulation of *CDC6* and other androgen-activated genes. In addition, it will be interesting to study in androgen-refractory PCa cells when androgen loses its activity, how FOXM1 is involved in the regulation of *CDC6* gene transcription. Furthermore, in other cancer types with the simultaneous overexpression of FOXM1, AR and CDC6, is the transcription of CDC6 regulated by FOXM1 and AR in a similar manner as we found in androgen-dependent PCa cells? These studies will provide more information about the dynamic tumor/tissue-specific transcription regulation controlled by multiple functional transcription factors.

Functionally, knockdown of FOXM1 and AR alone or in combination reduced DNA synthesis and cell proliferation in a manner that can be overcome through CDC6 overexpression. These data suggested that the collaboration of FOXM1 and AR transcription factors likely enhances DNA synthesis and accelerates cell proliferation by regulating CDC6 gene expression. Furthermore, siomycin A, a proteasome inhibitor known to inhibit FOXM1 expression and activity, inhibits prostate cancer proliferation and this effect is additive to that of the antiandrogenic compound bicalutamide. Revealing the transcription regulatory mechanisms of key oncogenes in specific cancer types will accelerate the development of novel personalized anti-cancer therapeutic modalities.

## Conflict of interests

The authors declare that there is no conflict of interests.

## Acknowledgements

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