The Circadian–Spliceosome Axis: A Temporal Dimension of Transcriptomic Heterogeneity in Prostate Cancer

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Abstract: Molecular heterogeneity and therapeutic resistance drive treatment failure in advanced prostate cancer. Although alternative splicing and transcript isoform remodeling—exemplified by the resistance-associated androgen receptor variant AR-V7—are critical components of this plasticity, the upstream dynamic mechanisms governing transcript-level diversification remain poorly understood. In this perspective, we propose a novel conceptual framework: the circadian–spliceosome axis. We hypothesize that core circadian networks, including BMAL1, CLOCK, PER, and CRY, act as higher-order temporal organizers that modulate RNA-processing machinery, enabling tumor cells to dynamically adopt non-genetic adaptive transcriptomic states under therapeutic pressure. Investigating this temporal dimension will require the integration of long-read sequencing, single-cell transcriptomics, and longitudinal sampling. If experimentally validated, the circadian–spliceosome axis could expand current models of non-genetic tumor adaptation and provide new opportunities for chronobiology-informed precision oncology aimed at disrupting the emergence of resistance-associated phenotypes.

Keywords: prostate cancer; circadian rhythm; alternative splicing; AR-V7; transcriptomic heterogeneity; precision oncology

Introduction

Prostate cancer is a biologically and clinically heterogeneous disease, encompassing tumors with markedly different molecular profiles, evolutionary trajectories, treatment sensitivities, and clinical outcomes. This heterogeneity is evident at the genomic, transcriptomic, proteomic, and phenotypic levels and contributes to a broad spectrum of clinical behavior, ranging from indolent localized disease to aggressive metastatic castration-resistant prostate cancer. Patients with apparently similar clinicopathological characteristics may respond differently to the same systemic therapies or develop resistance at different stages of disease. Although contemporary risk stratification integrates prostate-specific antigen levels, histopathological grade, disease stage, molecular features, and imaging findings, it cannot fully predict the course of an individual tumor or its response to treatment.

Genomic alterations account for an important component of this variability, but the tumor genome alone does not explain the dynamic and potentially reversible phenotypic changes that emerge during disease progression and under therapeutic pressure. Increasing evidence indicates that transcriptomic regulation, including alternative precursor messenger RNA (pre-mRNA) splicing, contributes to tumor adaptation by enabling cancer cells to modify their functional programs without requiring additional changes in the underlying DNA sequence. Through alternative splicing, a single gene can generate multiple messenger RNA and protein isoforms with distinct and occasionally opposing biological functions. Consequently, tumors with broadly similar gene-expression profiles may still exhibit functionally meaningful differences in transcript usage.

A clinically relevant example is androgen receptor splice variant 7 (AR-V7), which lacks the ligand-binding domain while retaining constitutive transcriptional activity. The detection of AR-V7 in circulating tumor cells has been associated with a reduced response to enzalutamide and abiraterone in patients with metastatic castration-resistant prostate cancer [1]. Nevertheless, the regulatory mechanisms that initiate, sustain, or modify such splicing events during disease evolution and under therapeutic pressure remain incompletely understood.

This perspective article proposes a potentially important but insufficiently explored connection between the circadian clock and the molecular machinery responsible for pre-mRNA splicing. This proposed relationship is referred to as the circadian–spliceosome axis. The central hypothesis is that the circadian system may influence not only the abundance and temporal expression of cancer-related genes but also the selection of the transcript isoforms produced from those genes. This distinction is biologically important because changes in isoform usage can alter protein structure, function, localization, molecular interactions, and downstream signaling without requiring additional genomic alterations.

Core circadian regulators, including brain and muscle ARNT-like protein 1 (BMAL1), circadian locomotor output cycles kaput (CLOCK), period proteins (PER1–3), and cryptochrome proteins (CRY1–2), could influence alternative splicing through several convergent mechanisms. These may include the temporal regulation of RNA-binding proteins and spliceosomal components, modulation of transcriptional kinetics and chromatin accessibility, and circadian variation in cellular metabolic states that affect RNA processing [2]. Disruption of these regulatory signals in prostate cancer could therefore alter splice-site selection and favor transcript variants that support tumor-cell survival under therapeutic pressure.

According to this model, circadian dysregulation may not merely accompany tumor progression but could contribute to adaptive non-genetic plasticity by modifying the repertoire of functional RNA and protein isoforms available to malignant cells. This

possibility may be particularly relevant to the emergence or persistence of androgen receptor variants, including AR-V7, and to the development of resistance to androgen receptor-directed therapy.

The circadian clock is a cell-autonomous timing system that coordinates approximately 24-hour oscillations in transcription, translation, metabolism, DNA repair, and other essential cellular processes. Its molecular architecture is based on interconnected transcriptional–translational feedback loops. Within the core loop, the BMAL1–CLOCK heterodimer binds to E-box regulatory elements and activates the expression of clock-controlled genes, including the negative regulators PER1–3 and CRY1–2. Following their accumulation and nuclear translocation, PER and CRY proteins repress BMAL1–CLOCK activity, thereby generating rhythmic cycles of activation and inhibition. Additional feedback loops involving the nuclear receptors REV-ERB and ROR, together with the post-translational regulation of clock proteins, contribute to the stability and tissue-specific organization of these oscillations [3].

Although circadian regulation is commonly considered in terms of gene-expression levels, its influence extends to co-transcriptional and post-transcriptional processes. Several RNA-binding proteins, splicing-associated factors, spliceosomal components, and epigenetic regulators exhibit circadian variation in their expression or activity, providing a mechanistic basis through which the biological clock could influence RNA processing [2]. Circadian regulation of alternative splicing has been demonstrated experimentally in biological systems, with both endogenous clock signals and environmental timing cues affecting the temporal production of distinct transcript variants [4].

In prostate cancer, circadian disruption has also been examined in relation to androgen receptor signaling, hormonal therapy, and cellular senescence, suggesting that the clock intersects with pathways relevant to disease progression and treatment response [3,5]. However, these relationships have generally been investigated at the level of signaling activity, overall gene expression, or cellular phenotype. Although circadian regulation and alternative splicing have been investigated in cancer and prostate cancer through distinct lines of research [2,3,6,8], the specific contribution of circadian regulators to spliceosomal activity and transcript-isoform selection during prostate cancer evolution remains insufficiently characterized. This knowledge gap is important because changes in RNA processing may occur rapidly and could facilitate tumor adaptation under therapeutic pressure without requiring new genomic alterations.

This proposed interaction warrants systematic investigation because alterations in circadian signaling could influence several interconnected stages of pre-mRNA processing. Circadian disruption may affect splice-site recognition, exon inclusion or skipping, intron retention, and the relative abundance of alternative transcript isoforms, thereby modifying the stability, coding potential, and functional properties of the

resulting RNA and protein products [2,6]. These effects could arise through rhythmic changes in the expression, activity, post-translational modification, or subcellular localization of splicing factors and RNA-binding proteins. They might also occur indirectly through alterations in transcriptional elongation, chromatin organization, cellular metabolism, or stress-response pathways.

Such mechanisms could enable prostate cancer cells to generate different functional transcript repertoires without additional changes to their underlying DNA sequence, depending on their temporal and physiological state. Under the sustained selective pressure imposed by systemic therapy, isoforms that preserve proliferative or survival signaling could confer a transient adaptive advantage, leading to the preferential survival and subsequent enrichment of tumor cells expressing these isoforms. In the context of androgen receptor-directed therapy, this process could potentially favor the expression or persistence of resistance-associated variants such as AR-V7 and other truncated androgen receptor isoforms [7].

The proposed model does not assume that circadian dysregulation is the sole or principal cause of AR-V7 generation, nor does it exclude established genomic, epigenetic, and treatment-related mechanisms of resistance. Instead, it positions temporal regulation as an additional and potentially modifiable layer that may influence the occurrence and functional consequences of alternative splicing events. Interindividual or treatment-induced differences in circadian organization might therefore contribute to variation in the production of resistance-associated transcripts, although this possibility requires direct experimental confirmation. The circadian–spliceosome axis thus provides a biologically plausible and experimentally testable framework for investigating how temporal regulation may contribute to transcriptomic heterogeneity and adaptive therapeutic resistance in prostate cancer (Figure 1).

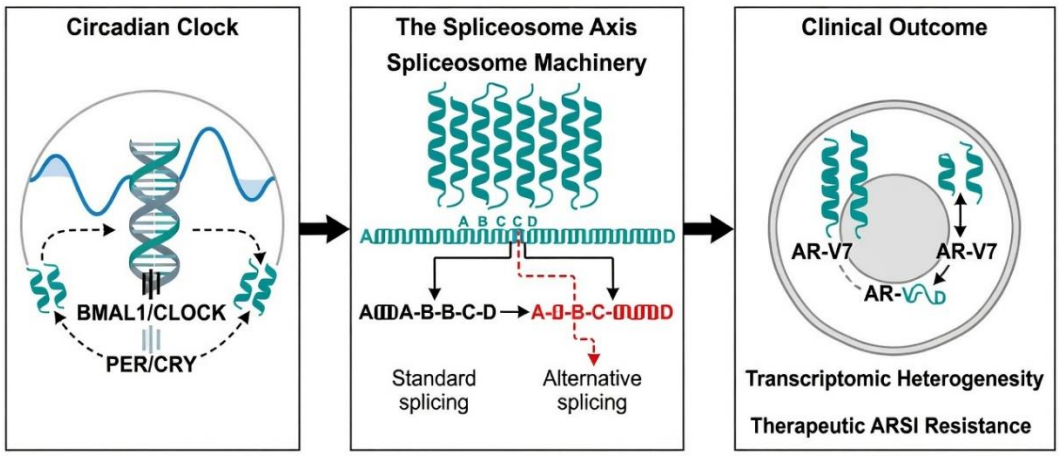

Figure 1. *Schematic presentation of the proposed circadian–spliceosome axis in prostate cancer, linking clock networks to alternative transcript usage and AR-V7-associated resistance.*

Future studies should evaluate this hypothesis using longitudinal and time-resolved experimental designs rather than relying exclusively on cross-sectional, single-time-point transcriptomic analyses, which may be confounded by biological phase and cannot adequately capture dynamic changes in RNA processing. The timing of biospecimen collection should therefore be recorded and, where feasible, standardized within study protocols. Samples obtained at different biological phases may differ in gene-expression levels and transcript-isoform abundance even in the absence of stable biological differences between tumors.

Serial blood-based sampling, including the analysis of circulating tumor cells or circulating tumor RNA, may offer a clinically feasible approach for distinguishing persistent resistance-associated splicing patterns from reversible temporal fluctuations. When repeated tumor biopsies are impractical, the clock time of sample collection, sleep–wake timing, treatment administration, and other relevant temporal variables should at least be documented to improve interpretation and reproducibility.

Long-read RNA sequencing would be particularly valuable for identifying full-length transcripts, complex exon combinations, and previously unrecognized androgen receptor variants that may be incompletely resolved by conventional short-read sequencing [8]. These analyses could be complemented by appropriately designed single-cell or single-nucleus approaches to determine whether clock-related transcriptional and splicing heterogeneity is associated with specific malignant or non-malignant cellular populations within the tumor microenvironment. However, isoform-level conclusions derived from short-read single-cell data should be interpreted cautiously and, where possible, validated using targeted assays or long-read sequencing.

Time-series datasets with adequate sampling density could subsequently be analyzed using established rhythm-detection methods, including cosinor-based approaches, to estimate the phase, amplitude, and reproducibility of temporal variation [9]. Together, these approaches would help determine whether the observed differences represent circadian or diurnal regulation, treatment-related adaptation, or stochastic variation in RNA processing. Controlled experimental protocols would be required to distinguish endogenous circadian regulation from variation driven by behavioral or environmental timing cues. Although such studies entail logistical and analytical challenges, incorporating biological time as a predefined experimental variable would substantially strengthen investigations of the proposed circadian–spliceosome axis.

Longitudinal collection of tumor tissue, circulating tumor cells, or circulating tumor-derived RNA before and during androgen receptor signaling inhibitor (ARSI) therapy could help determine whether changes in circadian organization and RNA splicing precede, accompany, or follow the emergence of resistance-associated transcripts. This temporal distinction would be important for differentiating potential predictive signals from downstream consequences of tumor progression. When serial tumor biopsies are not clinically feasible, repeated blood-based sampling may provide a less invasive approach for monitoring clock-gene expression, the abundance of candidate splicing factors, and androgen receptor isoform levels during treatment. Integrating these molecular measurements with prostate-specific antigen kinetics, imaging findings, treatment exposure, and clinical outcomes could identify temporal patterns associated with the development of resistance.

Complementary experimental studies should incorporate prostate cancer cell lines, patient-derived organoids, and in vivo models. Cell lines and organoids should undergo appropriate synchronization followed by time-resolved sampling, whereas in vivo models should be maintained under controlled light–dark and feeding schedules. Genetic perturbation of core clock components, together with carefully selected pharmacological modulation of circadian function, could then be used to assess their effects on spliceosomal activity, transcript-isoform selection, and sensitivity to androgen receptor-directed therapy. Relevant outcomes should include the abundance of AR-V7 relative to full-length androgen receptor, genome-wide alternative-splicing patterns, temporal changes in candidate RNA-binding proteins, and measures of treatment response.

Rescue experiments restoring individual clock components would strengthen causal inference and help distinguish specific clock-dependent effects from broader consequences of cellular disruption. Where possible, transcriptomic findings should also be validated at the protein and functional levels because changes in RNA isoform abundance do not necessarily translate into altered protein abundance or activity. Collectively, these approaches could clarify whether the proposed relationship is mediated by direct clock-dependent regulation of RNA processing, intermediate metabolic or epigenetic pathways, or broader treatment-induced adaptation. Assessing reversibility would be particularly important, as reversible temporally regulated splicing changes might represent therapeutically modifiable vulnerabilities.

Conclusion

The circadian–spliceosome axis remains a conceptual hypothesis, and direct experimental evidence supporting this relationship in prostate cancer is not yet available. Its validation will therefore require carefully controlled, time-resolved studies capable of distinguishing endogenous circadian regulation from treatment-related, environmental, behavioral, and stochastic variation in RNA processing. Nevertheless,

this framework raises biologically relevant and experimentally testable questions regarding how temporal organization may influence transcript-isoform selection, intratumoral heterogeneity, and the emergence of therapeutic resistance.

The proposed framework also extends the study of circadian disruption beyond changes in overall gene-expression levels by positioning alternative splicing as an additional layer of temporal regulation. A deeper understanding of the interaction between circadian biology and RNA processing may help explain how prostate cancer cells adapt at the transcriptomic level without acquiring new genomic alterations. If confirmed, this relationship could support the development of time-aware biomarkers, improve the interpretation of transcriptomic analyses, and encourage standardized reporting of biospecimen collection times in both experimental and clinical studies.

Ultimately, integrating biological time into investigations of RNA processing may reveal previously overlooked mechanisms of treatment adaptation and identify new opportunities for therapeutic intervention. The circadian–spliceosome axis should therefore be regarded not as an established mechanism but as a focused research direction warranting systematic investigation.

List of Abbreviations

ARSI, androgen receptor signaling inhibitor; AR-V7, androgen receptor splice variant 7; BMAL1, brain and muscle ARNT-like protein 1; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; DNA, deoxyribonucleic acid; PER, period protein; pre-mRNA, precursor messenger RNA; RNA, ribonucleic acid; ROR, retinoic acid receptor-related orphan receptor; REV-ERB, nuclear receptor subfamily 1 group D.

Author Contributions

Conceptualization, visualization, writing—original draft preparation, and writing—review and editing: I.B. The author has read and approved the final version of the manuscript.

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Conflicts of Interest

The author declares no conflicts of interest.

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AI-declaration

During the preparation of this manuscript, the author used ChatGPT (OpenAI) for language editing, rephrasing, and improving the clarity and organization of selected passages. The author critically reviewed and revised all AI-assisted content, independently verified the scientific claims and references, and accepts full responsibility for the integrity and accuracy of the final manuscript. No generative AI tool was used for the collection or analysis of research data.

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