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Multi-omics Analysis of Cell-Cycle Checkpoint Recovery after DNA Damage

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Abstract. Precise regulation of cell-cycle checkpoint recovery after DNA damage is essential for maintaining genome stability, and its dysregulation contributes to cancer therapy resistance. Recent advances in multi-omics technologies provide unprecedented opportunities to dissect this dynamic process; however, current studies often remain fragmented across individual omics layers. Here, we propose an integrated multi-omics framework for systematically analyzing checkpoint recovery. Guided by the temporal progression of activation–maintenance–restart, phosphoproteomics captures kinase dynamics, ubiquitinomics links phosphorylation to proteolysis, and transcriptomics reveals p53-mediated transcriptional programs. We further highlight the complementary roles of single-cell and spatial omics in resolving cellular heterogeneity and subcellular organization. Finally, we outline a closed "discovery–dissection–validation" workflow integrating CRISPR functional screens with multi-omics to bridge correlative observations and causal mechanisms.

Keywords: Cell-cycle checkpoint recovery, Multi-omics analysis, DNA damage response, Genome stability, Cancer therapy

1. Introduction

Cells have always been in the endogenous and exogenous nature of DNA damage, including ultraviolet radiation, ionising radiation, chemical mutants and reactive metabolic by-products. Insults can cause extensive DNA damage, including single-stranded fractures, double-stranded fractures, etc., causing long-term harm to genome stability. In order to ensure the integrity of the genome, cells will activate the DNA damage response (DDR), which is a very coordinated signal network that can detect DNA damage, transmit damage signals, and coordinate the repair process [1]. In addition to promoting DNA repair, DDR also combines with the cell cycle regulation mechanism to prevent damaged genetic material from being transmitted.

The main part of DDR is the activation of the cell cycle checkpoint, which temporarily stops the advancement of the cell cycle at the important transition points of G1/S and G2/M. Stop giving the repair a time window to ensure that the damaged DNA will not be copied or separated into the subcells. Checkpoint signals are mediated by phosphatidyl inositol 3-kinase-like kinase ATM and ATR, which activate the downstream effect factors CHK1 and CHK2. These kinases inhibit CDC25

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phosphatase, keep the CDK complex in an inactive state, and force the cell cycle to stop [2]. At the same time, the checkpoint signal was strengthened, and the stability of WEE1 kinase and the p53-dependent transcription program were activated.

Once the DNA repair is completed, the checkpoint signal must be terminated in order to return the cell cycle to its original orbit. This kind of transition is called checkpoint recovery, which is a highly regulated regulation process to kill DDR signals in a coordinated manner and reactivate mitotic kinase. Importantly, the timing of this transformation is also crucial. Starting over too early will make the genome unstable, mutations accumulate, and activation of checkpoints for a long time will cause irreversible results, such as cell ageing or apoptosis [3]. Therefore, checkpoint recovery is the decisive moment in determining whether the genome needs to be maintained and whether continuous cell proliferation should be maintained.

The recovery of checkpoints is regulated on multiple layers, namely kinase signalling, ubiquitin-mediated protein hydrolysis, transcription regulation, and chromatin remodelling. These regulatory processes do not exist in isolation, but are interconnected, forming a dynamic multi-level network. In this case, the multi-group method provides a powerful means to understand the checkpoint recovery mechanism at the system level [4]. The DNA damage in Figure 1 is activated by the ATM/ATR signal activation checkpoint, and then a series of coordinated molecular events are activated. Together, these molecular events promote the end of the checkpoint and start the restart of the cell cycle.

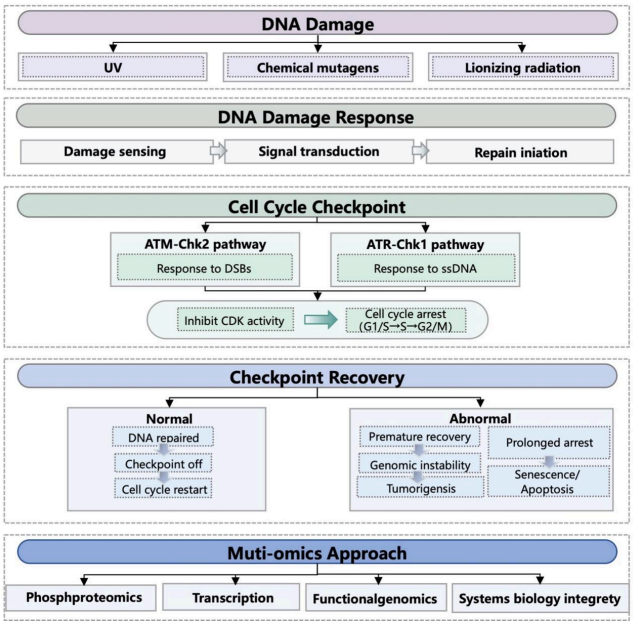

Figure 1. DNA damage response, checkpoint activation, and recovery, and the role of multi-omics technologies

2. Core regulatory mechanisms of checkpoint recovery

2.1. CDK1–Cyclin B1 and PLK1 as central drivers

The CDK1-Cyclin B1 complex is the main driver of the G2/M transition and the final execution module of checkpoint recovery. In the process of DNA damage, CDK1 activity is inhibited by the inhibitory phosphorylation of Tyr15 mediated by WEE1 kinase and CHK1/CHK2 signal inhibition

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of CDC25 phosphatase. Therefore, although the cyclic protein B1 protein continues to accumulate, the cells are still in the state of G2 stopping.

Checkpoint recovery is to reactivate the activity of CDK1-Cyclin B1. Quantitative proteomics shows that cyclic protein B1 still accumulates in large quantities during the stop period, forming an inactive complex library, which can be quickly activated during recovery. Phosphoproteinomic analysis shows that the inhibitory phosphorylation removal on CDK1 is highly nonlinear and switch-like, which is consistent with the outbreak of CDK1 substrate phosphorylation. This switch-like behaviour reflects that there is a strong positive feedback loop in mitosis entering the network. As long as the threshold is reached, a quick and irreversible commitment will be made to mitosis [5].

Polo-like kinase 1 (PLK1) will become the central coordinator of checkpoint recovery by regulating various pathways. PLK1 promotes checkpoint termination by phosphorylation of key inhibitory components, such as WEE1 and Claspin, so as to target ubiquitin-mediated degradation through SCFβ-TrCP E3 binding enzyme [6,7]. PLK1 activates CDC25 phosphatase, strengthening its ability to remove inhibitory phosphorylation from CDK1. Form a feedforward loop, that is, PLK1 eliminates the inhibitory signal on the one hand, and strengthens the mitosis-promoting signal on the other hand. After integrating phosphoproteomics and ubiquitin genomics data, it was found that PLK1 plays a regulatory role in many substrates involved in DNA damage signalling, cell cycle regulation and mitosis, indicating that it is not a single path element, but a systematic regulator.

2.2. Aurora A–Bora and spatial activation of PLK1

PLK1 is activated by upstream kinase, and the Aurora A-Bora complex plays a key role. According to phosphoproteomics research, in early recovery, a low level of CDK1 activity will promote Bora to be phosphorylated, thus binding to Aurora A, thus promoting the formation of active kinase complexes [8]. Form a hierarchical activation casde, that is, after the original CDK1 is activated by PLK1, it will continue to be amplified by PLK1.

Spatial proteomics and high-resolution imaging show that the activation of PLK1 mediated by Aurora A-Bora is done on the centre, thus forming a local signal hub. Space restrictions make the activation of PLK1 controlled and coordinated, and there will be no advance or abnormal activation of mitotic signals. Once activated, the PLK1 signal will be deployed throughout the body, resulting in a comprehensive reorganisation of the phosphorylated landscape. These findings show that spatial adjustment is important for checkpoint recovery, and there is also a spatial arrangement in subcellular compartments when kinase is activated.

2.3. WEE1 and CDC25 as checkpoint switch regulators

Between the stop of the checkpoint and the recovery, the balance of WEE1 kinase and CDC25 phosphatase plays a key role, and together they become the molecular switch that controls the activity of CDK1. WEE1 uses phosphorylation of CDK1 as an inhibitory regulator to maintain the state of the checkpoint. CDC25 eliminates this inhibitory phosphorylation and promotes mitosis.

The multi-omics analysis of integrated phosphoproteinomics and ubiquitin genomics shows that WEE1 is dynamically regulated by phosphorylation-dependent ubiquitinization. PLK1-mediated WEE1 phosphorylation produces identification signals, so that E3 ubiquitin binding enzyme can recognise it and degrade its proteasomes, thus removing the inhibition of CDK1 [9]. At the same time, CDC25 is activated by PLK1 and CDK1 phosphorylation to form a powerful positive feedback

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loop to accelerate the activation of CDK1. This kind of feedback-driven system ensures a fast and irreversible process from checkpoint interception to mitotic entry.

In addition, from the perspective of transcriptomics, p53 can regulate the expression of WEE1 and CDC25. Under the continuous DNA damage, p53 promotes the activation of inhibitors, which promotes the inhibition of the mitotic gene, thus strengthening the maintenance of the checkpoint. After the p53 signal decays, these transcription procedures will be reversed, thus promoting the restart of the cell cycle mechanism.

2.4. ATR/ATM–CHK1/2 and p53 signaling

The ATR/ATM-CHK1/2 signal axis is the centre where the checkpoint is activated and maintained, and the controlled termination is very important for the recovery of the checkpoint [10]. After DNA damage, ATR and ATM phosphorylate CHK1 and CHK2, thereby inhibiting CDC25 and stabilising WEE1 activity, thus forcing the cell cycle to stop.

The termination checkpoint signal relies on the coordinated dephosphorylation and degradation of DDR components. Phosphorus proteomics research has determined that protein phosphatase PP2A, PP1, Wip1/PPM1D, etc. are all regulatory factors for dephosphorylating ATM, CHK1/2 and downstream targets, resulting in the weakening of DDR signals [6]. The process of dephosphorylation is an important part of resetting the signal network and achieving recovery.

P53 is the central integrated element of the checkpoint signal, which plays a role in regulating transcription and post-translation response. When DNA is damaged, p53 will be phosphorylated and activated, which will promote the cell cycle to stop, DNA repair and the expression of genes related to apoptosis. When the checkpoint is restored, the activity of p53 is regulated by a variety of post-translation mechanisms, namely dephosphorylation, deacetylation and ubiquitination, thus reducing its transcription activity and stability[11] . In this way, cells can take into account the protection of the genome and the recovery of proliferation at the same time.

Overall, these findings show that the recovery of checkpoints is controlled by a highly coordinated network of kinase, phosphatase, ubiquitin-binding enzyme and transcription factors. The integration of multi-group methods can find these multi-layer regulation mechanisms, how the cell cycle re-enters the transition period after the checkpoint stops, and how time, space and function are coordinated.

3. Multi-omics technologies in checkpoint recovery research

3.1. Phosphoproteomics

The time-resolution phosphoprotein has become the main means of analysing the dynamic signal events behind the recovery of checkpoints. Using multiple time points to collect samples after DNA damage, kinase activity trajectory and large-scale identification of phosphorylation events can be obtained [12]. More importantly, phosphoproteomics can detect the activated state of CDK1 and PLK1, which are key kinases, and can also show the moment when its downstream substrate is activated, so as to establish a signal cascade system. Recent studies have combined phosphoproteomics with computational kinase activity prediction, making it feasible to identify known kinases involved in checkpoint recovery and kinases that have not been characterised before. These analyses illustrate the switch-like activation behaviour of mitotic kinase, and also give a quantitative understanding of how phosphorylation kinetics promote the transformation from checkpoint stop to mitotic entry.

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3.2. Ubiquitinomics and PTM-omics

Although phosphorylation plays a very important role in signal transmission, the recovery of the checkpoint also largely relies on the protein turnover mediated by ubiquitinisation. Ubiquitin genomics, especially the use of mass spectrometry to detect ubiquitin-modified peptides, can map protein degradation events and time dynamics globally [13]. The combination of ubiquitinology and phosphoproteomics shows that many checkpoint regulatory proteins are regulated by phosphorylation-dependent ubiquitination, thus linking kinase signals with protein hydrolysis. The phosphorylation of WEE1 and Claspin can promote the identification of E3 ubiquitin linkase, which in turn triggers proteasome degradation and promotes the end of the checkpoint. In addition to ubiquitinisation, other post-translational modifications such as acetylation and methylation also play a regulatory role in the stability and activity of proteins. PTM-omics gives a multi-level perspective of checkpoint recovery, showing how different types of modifications work together to fine-tune the regulatory network.

3.3. Single-cell multi-omics and spatial omics

The mass grouping is close to the average signal of the cell population, which will hide the intercellular heterogeneity that occurs during the recovery of the checkpoint. Single-cell multiomics technology, such as single-cell RNA sequencing and the latest single-cell proteomics, can deal with cell heterogeneity and identify various recovery pathways [14]. These methods show that even if the genes are the same, the response of cells to DNA damage will be different. Some will recover quickly, some will stop for a long time or even die. In addition, the combination of single-cell transcriptomics and living cell imaging makes there a direct correlation between molecular state and phenotytic behaviour.

Space omics technology can understand the subcellular tissue of the checkpoint signal. Important activation events can be discovered by drawing defined proteins and interactions in cell compartments, such as the activation of PLK1 mediated by Aurora A occurs in highly localised areas. This kind of spatial adjustment ensures the accurate coordination of the signal and highlights the necessity of the restoration of the compartment at the checkpoint.

3.4. CRISPR screens integrated with multi-omics

Using the whole genome CRISPR functional sieve, the gene that regulates the recovery of the checkpoint can be found. By linking CRISPR interference with phenotypic readings (cell cycle process or survival after DNA damage), we can systematically find out candidate regulators [15]. However, the insight into the mechanism obtained by relying only on CRISPR screening is limited. Combined with multi-group analysis, starting from the global signaling network changed after the disturbance of these candidates, these candidates can be characterised more comprehensively.

This combination strategy forms a powerful workflow. The CRISPR screen finds the functional regulator, and then does a omic analysis, such as proteomics or phosphoproteinomics, to explain the role of these regulators in the signalling pathway. This method greatly improves the discovery speed of the new regulator and deepens the understanding of the recovery mechanism of the checkpoint.

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4. Novel regulatory pathways revealed by multi-omics

4.1. Discovery of an ATM–E6AP–MASTL axis

The use of multi-group learning method system has found the supervision path for the restoration of checkpoints that have not been discovered before. The ATM-E6AP-MASTL axis links the upstream DNA damage signal with the downstream mitosis control [16]. In this pathway, ATM is activated after DNA damage, which causes the activity of E3 ubiquitin binding enzyme E6AP to increase, which in turn causes the ubiquitination and degradation of MASTL. Because MASTL inhibits phosphatase PP2A-B55, it increases phosphatase activity, thus maintaining a low level of CDK substrate phosphorylation and strengthening the stop of the checkpoint.

When the ATM signal weakens, E6AP is suppressed and MASTL is restored. MASTL can inhibit the activity of PP2A and increase the phosphorylation of CDK substrate, thus promoting mitotic entry. This method shows that the multi-omic method can reveal the coordinated regulation between cross-signal, ubiquitination and phosphatase activity, so as to fully understand the checkpoint control.

4.2. PHF6 as a novel regulator of checkpoint recovery

Another case of multi-omic-driven discovery was to use CRISPR to screen out PHF6 as a regulatory factor for checkpoint recovery. The loss of PHF6 will damage DNA repair and slow down the process of mitosis, which shows that it plays a certain role in the recovery of checkpoints. In terms of mechanism, PHF6 is a chromatin-related factor, which can promote the concentration of DNA repair proteins to the damaged site and accelerate the speed of repair.

After functional screening is integrated with proteomics and imaging analysis, it is clearly shown that PHF6 deletion can disrupt the key signalling pathways necessary for recovery. This example shows that chromatinal tissue plays an important role in checkpoint regulation, and also shows that it is valuable to integrate functional genomics with multi-omics to explore new biological mechanisms.

4.3. Multi-omics integration framework: from technical synergy to stage-wise division of labor

Figure 2 is a unified framework integrated with multi-group technology, which provides a systematic method for studying checkpoint recovery. The framework is built on the basis of the complementarity of different omology methods, and attaches importance to its coordinated use in time and space.

In terms of time, phosphoproteinomics has the highest amount of information when the early checkpoint is activated or restarted, because it can reflect the rapid change of kinase signalling at this time. Ubiquitin genomics is becoming more and more important in the recovery process. It shows the protein degradation events that drive the termination of the checkpoint. Transcriptomics provides a certain understanding of long-term regulatory procedures, especially those mediated by transcription factors (p53, etc.). Together, these methods can realise continuous tracking of the checkpoint dynamics from activation to mitotic re-entry.

In terms of space, single-cell and spatial omics expand the scope of analysis beyond the population average to solve cell heterogeneity and subcellular tissue. These techniques show that checkpoint recovery is not the same process. It is heterogeneous due to the influence of the intrinsic cell state and the local signalling environment.

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Most importantly, the integration based on CRISPR function screening has established a causal relationship within the framework. It combines discovery-driven screening with mechanical multi-group analysis, from identifying candidate regulators to understanding their role in complex networks. Finally, the multi-dimensional data set can be integrated using computational modelling and machine learning methods, which can realise the predictive behaviour of the system and turn static molecular data into a dynamic adjustment model.

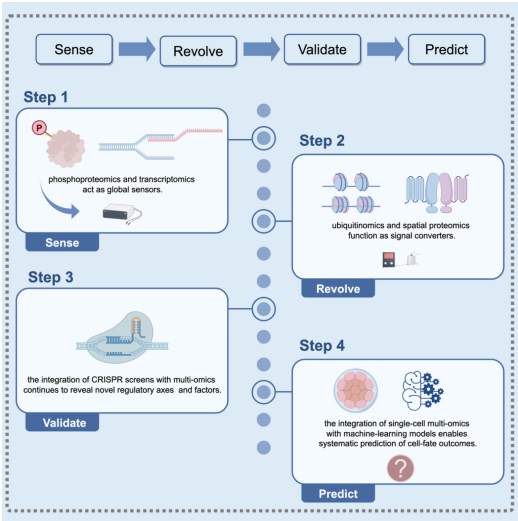

Figure 2. Integrated multi-omics framework for studying checkpoint recovery

5. Conclusion

The multi-group method fundamentally changes the study of cell cycle checkpoint recovery from the original linear path perspective to a dynamic system-level perspective. The integration of proteomics, transcriptomics, ubiquitinomics and functional genomics does not study a signalling component in isolation, but can comprehensively find the regulatory node and establish interaction between the multi-layer control layers. Especially in phosphoproteomics, it shows the time dynamics of kinase signals, ubiquitinomics links signal events with protein turnover, and transcriptomics shows long-term regulatory procedures triggered by transcription factors such as p53. Together, these complementary methods provide a multi-dimensional perspective on how cells coordinate the activation, maintenance and recovery of checkpoints.

The emergence of single-cell and spatial multi-omic technology, on the one hand, improves our understanding of checkpoint recovery by revealing cell heterogeneity and subcellular tissue, and on the other hand, it also gives us a more comprehensive understanding of checkpoint recovery. It can be seen from these studies that the recovery of checkpoints is not a unified process. It will be affected by the context, that is, the state of the cell, the metabolic status and the local signalling environment will affect its recovery. Based on CRISPR functional screening and multi-group integration, causal reasoning can be realised from related observations, so that important regulatory factors in complex networks can be systematically found and proved.

In the future, the research of checkpoint recovery will rely more and more on big data sets and high-dimensional data. Advances in computational modelling and machine learning will transform static comics data into predictive models that can express systematic behaviour and estimate cells' response to DNA damage. At the same time, the application of multi-group methods to more physiologically related systems, such as class and patient-derived models, can improve the

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transformation correlation of these findings. Finally, the system-level framework laid the foundation for the formulation of targeted treatment strategies that can regulate the recovery of checkpoints, improving the effectiveness of cancer treatment based on DNA damage and overcoming therapeutic drug resistance.

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