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Filling the mosaic of p53 actions

p53 represses RHAMM expression

The major challenge in understanding p53 function stems from the fact that it regulates a diverse array of cellular functions, including metabolism, DNA repair and stem-cell activity, some of which may not be directly connected with its ostensibly most important role—functioning as a key tumor suppressor protein. This difficulty is highlighted by findings of Sohr and Engeland described in the previous issue of *Cell Cycle*; they reported on p53's ability to repress expression of the RHAMM cell-surface and nuclear matrix protein, also known as CD168.[1]

RHAMM has at least two distinct functions. It operates as a cell-surface receptor for hyaluronan and a centrosomal protein that maintains stability of the mitotic spindle. Neither of these functions is crucial in development, since RHAMM-deficient animals develop normally. However, its functions appear to contribute to responses to pathological challenge, because RHAMM deficiency had been shown to impair wound healing.[2]

RHAMM is upregulated in many tumors, and its continued expression appears to be essential for the tumorigenicity of a number of cancer cell lines.[2] It shares these features with the CD44 transmembrane protein, which co-operates with RHAMM in a hyaluronan-dependent manner to promote cancer cell migration and invasion in some, but not all, experimental models of the disease.[2,3] Some have proposed that the apparently conflicting effects ascribed to CD44 in various experimental models of tumorigenesis may be due to the presence or absence of its RHAMM partner.[2] Ultimately, a more thorough evaluation of the interactions of these proteins should include analyses of yet other cell-surface receptors whose functions are directly stimulated by CD44, such as Her2 and cMet.[3]

Our own work recently demonstrated that p53 represses CD44 expression.[4] The very fact that both major hyaluronan-binding cell-surface proteins are repressed by p53 suggests that p53 has a more than a passing interest in blocking the signaling properties of the hyaluronan polymers present within the extracellular matrix. This ECM component is frequently expressed in the tumor-associated stroma and is known to promote various aspects of tumor growth.[3]

Previously reported evidence had suggested that RHAMM mRNA levels are suppressed by p53. As published in the previous issue of *Cell Cycle*, Sohr and Engeland demonstrate that the transcriptional activity of the RHAMM gene, as well as its mRNA and protein levels are regulated in a cell cycle dependent manner, albeit with slightly different kinetics. The usual transcriptional initiation site of this gene, as is the case with most genes, is nested in a promoter region located upstream of the translation initiation site. However, the RHAMM promoter site that is essential for a maximal response to p53-dependent repression is located downstream of the translation start site.[1]

It makes biological sense that the nuclear pool of RHAMM protein is expressed in a cell cycle-dependent manner, because RHAMM helps to maintain genomic stability and in this fashion acts as a tumor suppressor. But why then is its expression suppressed by p53, which itself is the archetypal tumor suppressor protein?[5] It is possible that the benefits from inhibiting the extracellular, clearly oncogenic function of RHAMM prevail over the price paid for inhibiting a potentially tumor-suppressing function of nuclear RHAMM.

If the degree of involvement of p53 in a particular biological function can be gauged by the number of interactions that it has with genes in a given pathway, then hyaluronan-dependent signaling and metabolism clearly emerges because of p53's involvement in repressing RHAMM and CD44.[1,4] The reasons why p53 represses CD44 expression are understandable: CD44 expression is known to counter p53-dependent functions such as inhibition of proliferation, inhibition of tumor growth, and induction of apoptosis.[4] Similar experiments with RHAMM still need to be done in order to gain a more definitive answer as to why p53 represses RHAMM expression. These results will need to be obtained from gain- and loss-of-function experiments that will examine the potential influences of both RHAMM and hyaluronan on various p53 functions, especially those that are relevant to its tumor-suppressing functions.

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Heterochromatin

Lost in Transcription?

Eukaryotic chromatin is typically divided into one of two domains: the loosely packed euchromatin and the highly condensed heterochromatin. Euchromatin is gene-rich and associated with active transcription. Heterochromatin is usually gene-poor and mainly composed of repetitive DNA sequences, which are often found near the centromeres and telomeres. While these DNA elements have long been regarded as the "junk" of the genome, heterochromatin is essential for repressing transcription and recombination at these regions to maintain genome integrity. Recent progress not only demonstrates that heterochromatin is a paradigm for the study of higher-order chromatin assembly, but also reveals heterochromatin as a highly dynamic structure that regulates diverse cellular processes.[1]

Post-translational modifications of histones, especially the methylation of lysines, play essential roles in heterochromatin assembly. For example, the methylation of H3K9 (histone H3 lysine 9) recruits HP1 proteins, which are essential for the maintenance of heterochromatin structures, whereas methylation of another histone lysine, H3K4, is excluded from this region1. While much has been elucidated about the role of histone methyltransferases in nucleation and maintenance of heterochromatin, the role of histone demethylases, which catalyze the removal of histone methylation, at this region still remain poorly understood.

In a paper published in this issue of *Cell Cycle*,[2] Pagano and colleagues discovered the requirement of the H3K36 demethylase KDM2A[3] for heterochromatin maintenance in mammalian cells. The authors found that KDM2A associated with all three HP1 variants and localized to pericentric heterochromatin. In addition, siRNA-mediated knockdown of KDM2A resulted in compromised heterochromatin function, such as delocalization of HP1 proteins, defects in chromosome segregation, reactivation of a heterochromatin-embedded reporter gene, and increased transcription of centromeric satellite repeats.[2]

The decrease of KDM2A resulted in an increase of H3K36 methylation (H3K36me) at heterochromatin.[2] H3K36me is generally associated with active transcription and recent studies in yeast demonstrate that it recruits a histone deacetylase complex to restore chromatin structures after transcription.[4] However, the function of this modification at heterochromatin is not clear. The identification of an H3K36 histone demethylase controlling heterochromatin stability adds a new twist to the already highly dynamic regulation of heterochromatin, and these results are consistent with recent studies demonstrating the role of

transcription in regulating heterochromatin stability.[1] Interestingly, overexpression of satellite repeats results in the delocalization of HP1,[2] suggesting that accumulation of these aberrant transcripts can directly cause heterochromatin defects, although the mechanism is not clear.

Until recently it was believed that there is little if any transcription at heterochromatin because it excludes the access of the general transcription machinery. However, the requirement of the RNAi pathway in heterochromatin assembly in diverse organisms promoted reexamination of transcription at heterochromatin.[1] Much of our understanding about this process comes from studies in the fission yeast *Schizosaccharomyces pombe*.[1] In this organism, transcription of repetitive DNA elements within heterochromatin generates substrates for the RNA interference (RNAi) pathway, which processes these transcripts into small RNA species referred to as small interfering RNAs (siRNAs) that target chromatin-modifying activities to heterochromatin regions. Interestingly, the transcription of DNA repeats is limited to the S phase of the cell cycle, concurrent with the loading of histone modifying enzymes to establish heterochromatin.[5,6] H3K36me levels also peak during the S phase together with increased levels of RNA polymerase II, and the H3K36 methyltransferase Set2 is required for heterochromatin assembly in fission yeast.[5] However, how this mark is removed after S phase is not clear. One possible contributor is a fission yeast homologue of KDM2A named Epe1 that associates with HP1 homologue Swi6.[1] Although no histone demethylase activity of Epe1 has been identified, mutations of key residues within the catalytic JmjC domain are nevertheless required for Epe1 function.[1] In mammals, the RNAi machinery is also required for heterochromatin integrity, and transcription of centromeric repeats is also cell cycle regulated.[7] Whether H3K36me levels at heterochromatin change during the cell cycle in mammals is currently unknown. Given the similarity of these processes between fission yeast and mammals, it is possible that KDM2A serves to demethylate H3K36me at heterochromatin after transcription to restore the original chromatin state.

In conclusion, these new findings expand our understanding of the physiological functions of histone demethylases. The discovery that KDM2A is a conserved regulator of heterochromatin stability provides new opportunities to study the role of transcription of repetitive DNA elements in heterochromatin maintenance.

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A novel MCM-2 fragment with potential biological function in senescence

This issue of *Cell Cycle* presents an important article on the identification of a novel MCM-2 fragment that may provide a clue to its regulation in senescing cells.[1] In their article entitled "Cleavage of MCM2 licensing protein fosters senescence in human keratinocytes", Harada et al. show that upon senescence of keratinocytes, a fragment of MCM2 is cleaved. This cleaved fragment is not found in cancer cells. The significance of this finding resides in the importance of MCM-2 in cell proliferation. MCMs (mini-chromosome maintenance proteins) are highly conserved in eukaryotes and required for initiation of DNA replication. The MCMs (2 through 7) form a hexameric protein complex which are necessary for the formation of the replication fork which together with ORC, Cdc6 and Cdt1 which interact with them, form the pre-replication complex. MCMs are specific markers of the cells cycle state in cells and tissues and are lost following differentiation and in quiescence state. As such, MCMs provide an attractive target for both prognosis and therapy in cancer. In fact in a recent clinical study it was shown that MCM-2 is a strong independent prognostic indicator in breast cancer.[2]

In the study of Harada et al.,[1] they show that a truncated form of MCM-2 with a molecular mass of 55 kDa is upregulated during replicative senescence, quiescence and differentiation. The generation of this fragment occurs post-transcriptionally and can be detected with an antibody targeted to the amino acids residues 131–150, but not to antibodies to the amino or carboxy terminus regions of the full length protein. Additionally the expression of this truncated MCM-2 is inversely correlated with the expression of the full length MCM-2 suggesting that the two may have opposing functions. While the precise function of the 55 kDa MCM-2 fragment is yet to be defined, its role in regulating the function of full length MCM-2 in pre-replciation complex could be important. For example, it is possible that once it is deciphered how this fragment is generated and if its generation is in fact correlated to a more differentiated state in tumor tissues, that its generation in tumor cells could be induced as a viable and tumor specific drug treatment. Additionally, with the current specific antibodies to both the full length and the 55kDa MCM-2 fragments, the importance of this truncated form of MCM-2 can be determined in normal, pre-malignant and malignant tissues.

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