At the Intersection of RNA Metabolism and Genome Maintenance in Cancer

NCI Workshop
December 6-7, 2021

Summary

Malignant transformation impairs cellular integrity on many levels. Cancer cells frequently exhibit both genomic instability and altered RNA metabolism and processing. Recent findings linking RNA metabolism to the DNA Damage Response (DDR) suggest that these two seemingly disparate processes may be functionally linked in tumorigenesis. Cancer-associated changes in RNA-processing factors or altered RNA post-transcriptional modifications can result in defective RNA maturation, stability and function, affecting the integrity of coding and non-coding transcriptomes. RNA processing defects have also been linked to structural changes in the genome, such as the formation of RNA:DNA hybrids (R loops), which can pose a threat to genome stability. Finally, RNA is emerging as a modulator of the DNA damage response through pathways involving preexisting transcripts at DNA damage sites, DNA damage-induced and damage-proximal transcripts, non-coding RNAs acting both in cis and trans to sites of damage, and DDR-induced changes in gene expression, RNA stability and RNA modifications.

On December 6-7, 2021, the National Cancer Institute (NCI) held a virtual workshop entitled “At the Intersection of RNA Metabolism and Genome Maintenance in Cancer” to explore alterations in RNA metabolism as a novel means to modulate genome stability and identify gaps and opportunities in the field. Participants included approximately 20 invited speakers from institutions across the US, Europe and Asia, as well as members of NCI Program staff.

The overarching goals of the workshop were (i) to explore emerging connections between the two historically distinct research areas of RNA metabolism and genome maintenance; and (ii) to bring together RNA-focused cancer biologists and genome maintenance experts to catalyze collaboration and explore alterations in RNA metabolism as a novel means to modulate genome stability and cancer progression. Talks and discussion were divided into three sessions reflecting the following complementary topics: RNA processing defects as drivers of genome instability (Session 1); RNA as a modulator of DNA repair (Session 2); potential therapeutic relevance of RNA metabolism for genotoxic therapy (Session 3). A summary of key conclusions and advances follows below.

Session 1: “RNA processing defects as drivers of genome instability” moderated by Dr. Karlene Cimprich

Session 1 was focused on understanding how RNA metabolism can interfere with genome maintenance and thereby contribute to genome instability. Several talks focused on adverse effects of R loops on the genome. Emerging evidence points to multiple mechanisms by which these hybrid nucleic acid species can harm the genome. R loops are perhaps best studied in the context of nuclear DNA, where they present obstacles to DNA transactions such as replication and transcription, often resulting in nuclear DNA damage. Recent work from Dr. Kathleen Burns from the Dana-Farber Cancer Institute and colleagues identifies transposable elements, long interspersed elements (LINEs), as a previously unappreciated sources of RNA-DNA hybrids attached to chromatin, the resolution of which appears to depend on a Fanconi Anemia-related repair mechanism (PMID: 32042151). Using an inducible R loop system, the

Paull lab at the University of Texas at Austin showed that the Double Strand Break (DSB) resection factor CtIP counteracts R loops through mechanisms that remain to be fully explored (PMID: 30523780).

Several exciting advances presented in this session extend the detrimental impact of R loops beyond the nucleus, involving RNA:DNA hybrid accumulation in the cytoplasm and mitochondria (Dr Ashok Venkitaraman, PMID: 34348152). The physiological significance and molecular basis of these observations will likely be a major focus of future research in this field.

To better understand R loop formation and resolution, Dr. Dirk Remus' lab at Memorial Sloan Kettering Cancer Center (MSKCC) has designed an in vitro system to reconstitute potentially harmful R loop:replisome collisions with purified proteins, which can further be combined with high resolution electron microscopy (PMID: 34494544). This approach provides a much-needed first step towards the detailed mechanistic dissection of R loop metabolism. Initial analyses identify distinct roles for G-quadruplex (G4) secondary structures and RNase H-mediated degradation in R loop resolution.

At least a subset of R loops result from aberrant RNA processing, such as mRNA splicing errors. Cancers with splice factor mutations exhibit elevated R loop levels, which in turn can introduce cancer cell-specific therapeutic vulnerabilities (PMID: 31080550). However, R loops are not the only means through which cancer-associated splice factor mutations can be exploited therapeutically. Work from the Abdel-Wahab lab at MSKCC provides an intriguing proof of principle for a strategy that takes advantage of synthetic introns specific for splice factor variants to selectively activate lethal genes in cancer cells. While preliminary, this work is likely to have exciting therapeutic implications.

Additional unexpected roles for RNA metabolism in genome maintenance were identified through studies of DSB repair by non-homologous end-joining (NHEJ) and transcription-coupled nucleotide excision repair, underlining that R loops are not the only means through which RNA metabolism can manipulate genome integrity.

Session 2: "RNA as a modulator of the DNA damage response" moderated by Dr. Andre Nussenzweig.

Session 2 highlighted the beneficial contributions of RNA metabolism in DNA repair and genome maintenance. RNA can directly contribute to DNA repair through a structural repair-promoting role at the site of DNA lesions. Conversely, DNA damage can regulate the transcription of repair-relevant transcripts through the formation of nuclear sub-compartments termed DNA damage or "D" compartments, as reported by Dr. Gaelle Legube from the University of Toulouse, France. Recent work from the d'Adda di Fagagna lab at the FIRC Institute of Molecular Oncology in Milan, Italy suggests an important role for telomeric non coding RNA at dysfunctional telomeres, with implications for diseases linked to telomere shortening and damage (PMID: 34680452). Mechanistic roles for break-associated RNAs are actively being investigated by multiple groups. Dr. Francesca Storici from Georgia Tech described a template or bridging role for RNAs complementary to DNA DSB ends, which promotes break repair in a sequence-dependent manner. DNA damage associated RNAs and their functions in DNA repair can be modulated by post-transcriptional RNA modifications, most notably 5-methyl cytosine, which is proposed to stabilize RNA:DNA hybrid formation observed at DNA breaks and provide a platform for the recruitment of homologous recombination factors. Dr. Li Lan from Massachusetts General Hospital described how RNA modifications influence

DNA repair pathway choice, and how they can be exploited for the treatment of HR-deficient tumors (PMID: 32503981). Together, these findings suggest that RNA at the site of DNA damage acts in a similar manner to DNA damage-induced chromatin modifications, which facilitate and orchestrate repair processes. Chromatin modifiers have been targeted in the context of genotoxic therapy. RNA, through its unique structures and modifications, may present additional therapeutic targets. One intriguing possibility is that manipulating RNA:DNA hybrid resolvases, such as RNase H1, could provide novel therapeutic strategies.

Finally, RNA is itself subject to damage, such as alkylation, which can impact the effectiveness of alkylation chemotherapy. Recent advances in understanding the RNA alkylation repair machinery spearheaded by Dr. Nima Mosammaparast at Washington University in St. Louis and colleagues may help uncover synthetic vulnerabilities to improve cancer therapy.

Session 3: "Exploiting RNA metabolism in cancer and genotoxic therapy" moderated by Dr. Lee Zou

Session 3 focused on ongoing efforts to target RNA metabolism to improve personalized genotoxic therapy. Consistent with the previous sessions, R loops emerged as a central means to manipulate genome integrity in cancer cells. The Zou lab identified R loops as a potential vulnerability in cancers that depend on Alternative Lengthening of Telomeres (ALT), a process that has been associated with excessive R loop formation (PMID: 33453166). Mechanistic insight into the ALT factors that link R loops to telomere extension is expected to identify novel therapeutic targets in these aggressive tumors. Highlighting the potentially broad applicability of R loops as a therapeutic target, speakers described their potential as an exploitable vulnerability in multiple cancers with aberrant transcriptional programs, such as MYC-overexpressing tumors and Ewing Sarcoma (PMID: 30894746, PMID: 29513652). In MYCN-driven neuroblastoma, oncogenic transcription factor activity causes excessive transcription:replication conflicts, which can be partially ameliorated through exosome-mediated transcript clearance, suggesting yet another R loop-related therapeutic vulnerability. Together, these findings underline the need for improved computational strategies to map and define R loops, particularly in heterogeneous cancer cell populations. Limitations of commonly-used mapping methods were apparent in comparative reanalysis of existing data sets. A more comprehensive approach called ChAR-Seq to map chromatin-associated RNAs, including but not limited to R loops, may hold promise for better understanding the landscape and function of DNA- or nucleosome-associated RNAs (PMID: 29648534).

A genetic approach to dissect R loop contribution to cancer was presented by Dr. Rong Li, George Washington University, who used a mouse model to assess mammary epithelial tumor formation in the absence of BRCA1, which has been associated with increased R loop formation. Remarkably, early data suggest that a genetic rescue of increased R loop formation does not affect mammary tumorigenesis. While more work is needed to conclusively address this issue, these findings bring into question to what extent R loops promote malignant transformation.

Another compelling finding, from Dr. Dipanjan Chowdhury at Dana-Farber Cancer Institute, was that deletion of the RNA-binding DNA repair protein TIRR offered protection from tumorigenesis in mice heterozygous for the tumor suppressor p53. Suggesting relevance for human disease, low TIRR levels correlate with improved survival in patients with Li-Fraumeni Syndrome. Whether or not this phenomenon is due to TIRR interaction with RNA, and/or a role for RNA in DNA repair remains to be determined.

Summary and open questions

This workshop emphasized the importance of RNA metabolism for a diverse range of genome maintenance pathways, many of which have bearing on disease. The most extensively investigated link between RNA and genome instability relates to the formation and resolution of R loops. A significant body of work exists establishing R loops as both effectors of and obstacles to genome integrity. Data presented over the course of the workshop highlighted the emerging importance of R loops beyond the nucleus, with the potential to trigger DNA damage-associated immune activation and mitochondrial dysfunction. Associations between R loop formation and disease, particularly malignant transformation, were abundant. More research is needed to determine whether these associations are causal or merely correlative. Even if R loops are not causal, they may still be therapeutically valuable as a cancer vulnerability, and thus may present an important opportunity for the development of targeted therapeutic approaches.

The plethora of distinct effects attributed to R loops was striking. Future research is needed to understand the diversity of R loops and distinguish between their functions in various processes such as DNA replication, transcription, mRNA processing and innate immune sensing. It further remains unclear what sets apart beneficial R loops from those with adverse effects on cell and nuclear function. The development of new tools will be essential for the advancement of research in this area. This includes new strategies allowing for targeted manipulation of specific R loop subsets, locus-specific assays that monitor R loops at single-cell resolution over time, and novel genome-wide assays. The currently available tools to interfere with or manipulate R loops, such as RNase H1 overexpression, are blunt and may be insufficient to study their physiological relevance in health and disease.

While R loops were an omnipresent theme, RNA and its ribonucleotide building blocks are now implicated in a growing number of genome maintenance aspects, from bona fide repair processes such as NHEJ and microhomology-mediated end-joining to serving as a source of damage that can be a cancer vulnerability itself, such as alkylated RNA species. Moreover, the process of transcription causes DNA damage independent of R loops both through known pathways and yet-to-be determined mechanisms that lead to single-stranded DNA lesions in transcribed regions. The latter was highlighted in the Keynote lecture by Dr. Andre Nussenzweig, who employed creative genome-wide approaches to map the appearance of single-stranded DNA lesions, a frequent occurrence in postmitotic neurons, to active enhancers. While transcription of these enhancers and associated enhancer RNAs appear to promote these lesions, the molecular basis for this intriguing observation remains unclear.

Overall, future research efforts are expected to focus on two major themes: (i) building on existing observations to demonstrate functional relevance for RNA metabolism, and particularly R loops, in disease; and (ii) development of new tools to better understand the dynamics and specificity of RNA-associated genome maintenance.