Potential Roles of Long Non-Coding RNAs (lncRNAs) in Stress Response Regulation

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INTRODUCTION

It was previously thought that proteins are responsible for the transaction of genetic information. Still, lately, there have been substantial shreds of evidence to support the pervasiveness of transcription throughout the eukaryotic genome resulting in numerous key regulatory non-coding RNAs or ncRNAs (Wilusz et al., 2009). Even though about 90% of the human genome is actively transcribed, only about 20,000 proteins are encoded, which is below 2% of the total genome sequence (Gibb et al., 2011). Therefore, the human transcriptome is now considered to be much more complex and complicated than what was previously presumed. The ‘dark matter’ or the once considered to be transcriptional noise has been shown to participate in various significant biological roles (van Bakel et al., 2010). The ncRNAs are functional RNA transcripts for gene regulation at the level of transcription and post-transcription. There have been vital pieces of evidence to support the role of regulatory non-coding RNAs in the eukaryotic genome in recent years. The ncRNAs are also associated with post-translational modifications such as histone modification, heterochromatin formation, DNA methylation and other key molecules which are involved in regulating chromatin structures for gene expression. LncRNAs (long non-coding RNAs) are the most diverse, biologically active transcripts without significant open reading frames (ORFs) and represent the majority of ncRNAs populations in the human genome. Emerging pieces of evidence suggest the role of ncRNAs in a wide range of human diseases, including cardiovascular, Alzheimer, and cancer. Several reports in the recent past also supported their involvement in the modulation of various cellular responses, although the mechanisms of ncRNAs mediated gene regulations are still not fully understood. This review paper highlights the importance of lncRNAs in cellular stress response such as DNA damaging ionizing radiation that will encourage research in thrust areas of therapeutics and diagnostics. The involvement of important lncRNAs in regulating biological processes, responses to ionizing and non-ionizing radiation, as well as methods for the analysis of their cellular expression has been discussed.
post-transcription. The ncRNAs are also associated with post-translational modifications such as histone modification, heterochromatin formation, DNA methylation and other regulators of gene expression. The ncRNAs are broadly classified into short ncRNAs, which are less than 30 nucleotides as well as long ncRNAs (longer than 200 nucleotides). The short ncRNAs can be further classified as miRNAs, piRNAs and siRNAs. The binding of miRNA to a complementary mRNA target leads to degradation or inhibition of translation.

Similarly, siRNAs (short interfering RNAs) act as gene silencer at the post-transcription level by cleavage of mRNA targets. Another type of miRNAs, piRNAs or Piwi-interacting RNAs interact with proteins which are involved in regulating chromatin structures. Therefore, the regulatory implications of ncRNAs may have significant roles in diagnostic and clinical applications. The role of miRNAs in cellular responses to stress, such as ionizing radiation, has also been reported (Chaudhry, 2014). The level of plasma miRNA expression was found to be dose-dependent in mice that received total body irradiation (Chaudhry, 2014; Cui et al., 2011). Therefore, miRNAs have a promising role as biological markers since radiation exposure is one area of significant relevance to human health and environment, identifying and understanding these non-coding RNAs in radiation-related stress responses would be highly beneficial in the development of diagnostic and dosimetry tools for radiological emergencies.

LncRNAs are biologically active transcripts having more than 200 nucleotides which lack significant ORFs. A list of different types of human lncRNAs is given in Table 1 (Gibb et al., 2011). LncRNAs usually represent intronic or intergenic sequences which may be transcribed in the opposite direction to genes that encodes protein. These endogenous cellular RNAs are found to regulate gene expression at the level of chromatin, transcription and post-transcription. They can be further classified as intronic, anti-sense, and long intervening ncRNA (lincRNA) that neither overlaps exons nor other lncRNAs. One example of lincRNAs is Xist (X-inactive specific transcript gene) known to be associated with chromatin modifications via methylation of histones and chromosome inactivation (Kaikkonen et al., 2011). LncRNAs are the most diverse and represent the majority of the non-coding RNAs populations in the human genome. Their regulatory roles have been reported at the level of epigenetics, transcription, translation, RNA processing and modification (Hall et al., 2015). The involvement of lncRNA in several important cellular regulatory roles and are also implicated in various human diseases. However, studies on the lncRNAs regulatory role and mechanism in gene expression are still limited.

Nevertheless, evidences are emerging on the significant contribution of lncRNAs in DNA damages, radiation responses, human diseases, and other stresses. The potential use of lncRNA as biomarkers is now strongly substantiated in recent reports for it’s used in different types of cancer (Peng et al., 2020). Here we highlight some important findings that lncRNAs may play a significant role in cellular stresses.

**Role as an Indicator of Biological Processes**

There has been increasing evidence implying lncRNAs regulatory role related to biological processes. Substantial evidences have linked lncRNAs to cancer development (Gibb et al., 2011). The mode of gene regulation can be manifold since lncRNAs are known to interact with proteins as well as RNAs, such as the regulation of gene expression due to DNA damage responses. For example, the activation of PANDA and ANRIL expression was aided by p53 (Hung et al., 2011) and a transcription factor E2F1 (Wan et al., 2013), respectively. Recent reports revealed the role of Trp53cor1 (lincRNA-p21) in apoptosis and preventing cell proliferation by downregulating several p53 genes following irradiation exposure (Beer et al., 2017). Analysis of the lncRNA expression will yield new information that will help understand stress responses.

**Role in Stress Response towards Ionizing Radiation**

The effect of a high dose of radiation on PBMCs as well as the role of lncRNAs remains unclear. It was shown that a high dose of irradiation (≥30 Gy) was necessary for the cleavage of caspase-3-dependent apoptosis with the regulation of approximately 10% of lncRNAs in PBMCs at 20 h post-irradiation period (Beer et al., 2017). Besides, several lncRNAs were also reported to be potential candidates of irradiation response. This study employed a maximum dose of up to 60 Gy on PBMCs. Another possible role of lncRNA is inhibition of miRNA binding to its target mRNA (Ulitsky and Bartel, 2013). Irradiation induced upregulation of lncRNAs resulted in repression of p53 interacting/inhibiting miRNAs resulting in derepression of p53. It was also shown that lncRNAs coordinate with mRNAs and miRNAs in PBMCs following high radiation exposure providing a new therapeutic approach for cancer (Beer et al., 2017). Ionizing radiation also affects the regulation of Cyclin D1 (CCND1) through ncRNA-CCND1 (Wang et al., 2008). High dose irradiation (5 Gy) results in the overexpression of ANRIL (anti-sense non-coding RNA in
Table 1: Examples of human lncRNA*

| Name                                      | Symbol       |
|-------------------------------------------|--------------|
| Long stress-induced non-coding transcripts| LSINCTs      |
| Long or large intergenic ncRNAs           | lincRNAs     |
| Transcribed ultraconserved regions        | T-UCRs       |
| GAA-repeat containing RNAs                | GRC-RNAs     |
| Ribosomal 18S and 28S RNAs                | rRNAs        |
| Promoter-associated long RNAs             | PALRs        |
| Pseudogenes                                | None         |
| Stable excised intron RNAs                | None         |
| Long intronic ncRNAs                      | None         |

* (Gibb et al., 2011)

the INK4 locus), that may lead to p16 inhibition of senescence (Özgür et al., 2013).

In a study, the expression of PARTICLE (promoter of MAT2-antisense radiation-induced circulating lncRNA) was increased significantly following radiation exposure ranging from 0.25 to 2.5 Gy with 0.008 Gy/s (O’Leary et al., 2015). PARTICLE and MAT2A transcripts co-localized in the cytosol and possibly for exportation via exosomes following irradiation. MAT2A transcription started earlier in less than 4 hr following low dose irradiation resulting in accumulation of SAM levels. Genotoxic stress such as irradiation leads to the production of SAM due to its methyl donor activity for detoxifying methylation reactions. Therefore, the function of PARTICLE could be controlling the level of methyl groups for DNA damage repair.

Role in Stress Response towards Non-Ionizing Radiation

LincRNA-p21 (long intergenic non-coding RNA) is found to play an important role in apoptosis after exposure to UV-B (Hall et al., 2015). It was demonstrated that UV-B exposure leads to lincRNA-p21 regulation at the transcriptional level involving the p53 pathway. Different stress signals induced a type of lncRNA called PRINS, including UV-B irradiation at 110 mJ/cm² on human keratinocyte cell line HaCaT (Sonkoly et al., 2005).

The possible regulatory role of MALAT1 in UVB-induced photo-ageing was also reported (Lei et al., 2017). In this study, 60 mJ/cm² UV-B irradiation was sufficient to induce high-level expression of MALAT1 in fibroblasts. It was also proposed that MALAT1 expression in response to UVB is independent of ROS production with -acetyl-L-cysteine. MALAT1 has been known to play an important role in several human diseases such as cancer (Wei and Niu, 2015).

Methods for the analysis of LncRNA expression

Recently ENCODE (Encyclopedia of DNA Elements) project reported 15,512 transcripts grouped into nearly 10,000 lncRNA gene loci (Derrien et al., 2012). The identification and characterization of gene regulatory lncRNAs have been achieved successfully by techniques including tiling array, microarray, immunoprecipitation of RNA and chromatin, RNA-seq, knockdown, In situ hybridization, northern blot and pull-down analysis (Lee and Kikyo, 2012; Feng et al., 2014). RNA immunoprecipitation (RIP) a common technique for enrichment of lncRNAs may involve cross-linking. This technique primarily requires the immunoprecipitation of RNA binding proteins and the subsequent identification of the RNA bound to these proteins. Soluble lncRNA complexes that can be easily separated from the chromatin can be employed without cross-linking. RIP technique, in combination with microarray or RNA-seq analysis, can also be helpful when dealing with unknown lncRNAs (Zhao et al., 2010).

The main advantage of RIP technique is the identification of lncRNA bound to the target protein. At the same time, the use of pull-down ensures the identification of protein molecules that interact with a specific lncRNA. Northern blot analysis will help in determining the lncRNA abundance as well as the lncRNA spliced variants. In situ hybridization of lncRNA will help determine the localization and expression level of the desired lncRNA (Feng et al., 2014). RNA-seq is a highly advanced and efficient technique working on a genomic scale of single-base resolution. It can be used for detection of already known as well as unknown lncRNAs (Atkinson et al., 2012). The main disadvantages of RNA-seq are the high cost, complexity and the time consumed in data analysis (Lee and Kikyo, 2012). Recently, a technique called ChIPR-MS (Comprehensive Identification of RNA-binding proteins by mass spectrome-
try) was optimized for the identification of IncRNA-bound proteome (Chu et al., 2015).

On the other hand, micro-array-based tools provide a better alternative for several applications. However, microarrays are not suitable for detection of a novel or unknown IncRNAs. In such cases, special probes with exon-exon boundaries are required for detection of different splicing variants of IncRNAs from the RNA pool. Another useful technique is DNA tiling arrays for the identification of novel IncRNAs without prior knowledge of their exact location within a given DNA region at a high resolution (Lee and Kikyo, 2012).

CONCLUSIONS

The involvement of IncRNAs in gene regulation and several human diseases has emerged with the recent advances in genomics and proteomics platforms. The potential role of IncRNA in cellular responses towards stress, including DNA damaging ionizing radiation will encourage research in thrust areas of therapeutics and diagnostics. More research will be necessary to understand the exact mechanisms of IncRNA expression and the various regulatory processes in unexplored in diverse organisms.

Conflict of Interest

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REFERENCES

Atkinson, S. R., Marguerat, S., Bähler, J. 2012. Exploring long non-coding RNAs through sequencing. *Seminars in Cell & Developmental Biology*, 23(2):200–205.

Beer, L., Nemec, L., Wagner, T., Ristl, R., Altenburger, L. M., Ankersmit, H. J., Mildner, M. 2017. Ionizing radiation regulates long non-coding RNAs in human peripheral blood mononuclear cells. *Journal of Radiation Research*, 58(2):201–209.

Chaudhry, M. A. 2014. Radiation-induced microRNA: Discovery, functional analysis, and cancer radiotherapy. *Journal of Cellular Biochemistry*, 115(3):436–449.

Chu, C., Spitale, R. C., Chang, H. Y. 2015. Technologies to probe functions and mechanisms of long non-coding RNAs. *Nature Structural & Molecular Biology*, 22(1):29–35.

Cui, W., Ma, J., Wang, Y., Biswal, S. 2011. Plasma miRNA as Biomarkers for Assessment of Total-Body Radiation Exposure Dosimetry. *PLoS ONE*, 6(8):e22988–e22988.

Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Dje-bali, S., Tilgner, H., et al. 2012. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. *Genome Research*, 22(9):1775–1789.

Feng, Y., Hu, X., Zhang, Y., Zhang, D., Li, C., Zhang, L. 2014. Methods for the study of long non-coding RNA in cancer cell signalling. In *Cancer Cell Signalling*, pages 115–143. Humana Press.

Gibb, E. A., Brown, C. J., Lam, W. L. 2011. The functional role of long non-coding RNA in human carcinomas. *Molecular Cancer*, 10(1):38–38.

Hall, J. R., Messenger, Z. J., Tam, H. W., Phillips, S. L., Recio, L., Smart, R. C. 2015. Long non-coding RNA lincRNA-p21 is the major mediator of UVB-induced and p53-dependent apoptosis in keratinocytes. *Cell Death & Disease*, 6(3):e1700–e1700.

Hung, T., Wang, Y., Lin, M. F., Koegel, A. K., Kotake, Y., Grant, G. D., et al. 2011. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nature Genetics*, 43(7):621–629.

Kaikkonen, M. U., Lam, M. T. Y., Glass, C. K. 2011. Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovascular Research*, 90(3):430–440.

Lee, C., Kikyo, N. 2012. Strategies to identify long noncoding RNAs involved in gene regulation. *Cell & Bioscience*, 2(1):37–37.

Lei, L., Zeng, Q., Lu, J., Ding, S., Xia, F., Kang, J., J. Z. 2017. MALAT1 participates in ultraviolet B-induced photo-ageing via regulation of the ERK/MAPK signalling pathway. *Molecular Medicine Reports*, 15(6):3977–3982.

O’Leary, V. B., Ovsepian, S. V., Carrascosa, L. G., Buske, F. A., Radulovic, V., Niyazi, M., Moertl, S., Trau, M., Atkinson, M. J., Anastasov, N. 2015. PARTICLE, a Triplex-Forming Long ncRNA, Regulates Locus-Specific Methylation in Response to Low-Dose Irradiation. *Cell Reports*, 11(3):474–485.

Özgür, E., Mert, U., Isin, M., Okutan, M., Dalay, N., Gezer, U. 2013. Differential expression of long non-coding RNAs during genotoxic stress-induced apoptosis in HeLa and MCF-7 cells. *Clinical and Experimental Medicine*, 13(2):119–126.

Peng, S., Yin, X., Zhang, Y., Mi, W., Li, T., Yu, Y., Y. F. 2020. Competing endogenous RNA network analysis reveals potential long non-coding RNAs as predictive biomarkers of gastric cancer. *Oncology Letters*, 19(3):2185–2196.
Sonkoly, E., Bata-Csorgo, Z., Pivarczi, A., Polyanka, H., Kenderessy-Szabo, A., Molnar, G., et al. 2005. Identification and Characterization of a Novel, Psoriasis Susceptibility-related Noncoding RNA gene, PRINS. *Journal of Biological Chemistry*, 280(25):24159–24167.

Ulitsky, I., Bartel, D. P. 2013. lincRNAs: Genomics, Evolution, and Mechanisms. *Cell*, 154(1):26–46.

van Bakel, H., Nislow, C., Blencowe, B. J., Hughes, T. R. 2010. Most "Dark Matter" Transcripts Are Associated With Known Genes. *PLoS Biology*, 8(5):e1000371–e1000371.

Wan, G., Mathur, R., Hu, X., Liu, Y., Zhang, X., Peng, G., Lu, X. 2013. The ATM-E2F1 signalling pathway induces long non-coding RNA ANRIL (CDKN2B-AS). *Cellular Signalling*, 25(5):1086–1095.

Wang, X., Arai, S., Song, X., Reichart, D., Du, K., Pascual, G., Tempst, P., Rosenfeld, M. G., Glass, C. K., Kurokawa, R. 2008. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. *Nature*, 454(7200):126–130.

Wei, Y., Niu, B. 2015. Role of MALAT1 as a prognostic factor for survival in various cancers: a systematic review of the literature with meta-analysis. *Disease markers*.

Wilusz, J. E., Sunwoo, H., Spector, D. L. 2009. Long noncoding RNAs: functional surprises from the RNA world. *Genes & Development*, 23(13):1494–1504.

Zhao, J., Ohsumi, T. K., Kung, J. T., Ogawa, Y., Grau, D. J., Sarma, K., Song, J. J., Kingston, R. E., Borowsky, M., Lee, J. T. 2010. Genome-wide Identification of Polycomb-Associated RNAs by RIP-seq. *Molecular Cell*, 40(6):939–953.