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Unveiling Cryptic Transcription: Loss of Transcriptional Fidelity in a Skin Cellular Aging Model during Senescence

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1. Introduction

Epigenetic mechanisms play a crucial role in regulating longevity and are recognized as a primary hallmark of aging [1, 2]. Chronological aging and the exposome can disrupt these epigenetic mechanisms, resulting in a loss of transcriptional fidelity, commonly referred to as 'cryptic transcription' [3]. Cryptic transcription occurs as a consequence of erroneous DNA compaction, which in turn facilitates hazardous transcription initiation. Such misregulation can lead to frame shifts during transcript production, ultimately resulting in the synthesis of dysfunctional proteins.

DNA methylation is the first epigenetic mechanism that plays a pivotal role in maintaining skin homeostasis. This methylation predominantly occurs at CpG sites and is mediated by

DNA methyltransferases (DNMTs) [4, 5]. In aging, a linear decrease in the expression of DNMT3B has been demonstrated, particularly in females, while DNMT1 shows a decline only after the age of 60 [6, 7]. This finding reinforces the major role of DNMT3B in maintaining epigenetic mechanisms throughout the entire lifespan.

Reduced methyltransferases cause chromatin compaction errors, leading to an increased number of frame shifts during transcription. This results in truncated transcripts, known as Incomplete Slice Matches (ISM), that code for dysfunctional proteins (Figure 1).

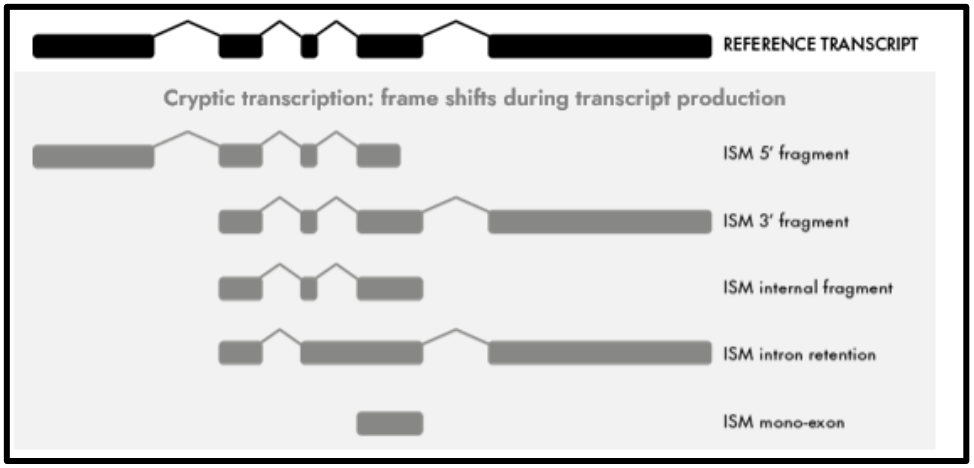

Figure 1. Examples of isoforms resulting from cryptic transcription (adapted from github.com). Reference Transcript refers to Full Splice Match, representing full-leght coding RNAs.

After their translation, these erroneous RNAs undergo the classical maturation steps, including 5’ capping and 3’ polyadenylation, and are ultimately translated into nonfunctional proteins. These classical maturation steps prevent dysfunctional transcripts from being recognized by the proteasome, leading to the accumulation of dysfunctional proteins within the cell.

This phenomenon of nonfunctional protein accumulation in aging is further exacerbated by the decreased activity of the proteasome with age [3, 8].

This study presents a novel methodology to investigate cryptic transcription in skin cells. Using next-generation sequencing, we analyze full-length transcripts from control and prematurely aged keratinocytes to unveil the mechanisms of cryptic transcription in senescence.

2. Materials and Methods

2.1. DNMT3B quantification and telomerase activity quantification

To validate the culture model prior to the sequencing experiment, the expression of DNMT3B and telomerase activity were measured.

DNMT3B was quantified using the ELISA method. It has been previously reported that senescent cells, which exhibit cryptic transcription, show reduced production of DNMT3B. Briefly, Normal Human Epidermal Keratinocytes (NHEKs) from a 50-year-old donor were cultivated in monolayer at passage 2 for 72 hours. These cells served as the non-senescent control. NHEKs from this same donor were then sub-cultivated until passage 4. At the end of the incubation period, nuclear proteins were extracted from the cell monolayer. DNMT3B was quantified using a sensitive and specific ELISA kit (Abcam, AB113471).

Telomerase activity was measured by qPCR (ScienCell Relative Human Telomere Length Quantification qPCR Assay), using NHEKs, following the same protocol as previously described.

2.2. Keratinocytes culture and RNA extraction for sequencing

For the sequencing study, a new culture of NHEKs was established using human keratinocyte cells from a 47-year-old donor. At the end of the incubation period, cell monolayers were then processed for RNA extraction using a dedicated RNA extraction kit (Qiagen).

The quality of the RNA was assessed using a Nanodrop spectrophotometer and the 4200 TapeStation (Agilent Technologies). A quality score (RIN) was calculated by the TapeStation, with samples required to achieve a RIN greater than 7 and a minimum concentration of 45 ng/µL. RNA preservation is crucial for performing a reliable analysis of full-length transcripts.

2.3. Full length RNA library preparation and sequencing Pacbio® sequencing technology

An Iso-Seq® protocol was employed and library preparation was conducted, which involved circularizing the cDNA using the 'SMRTbell® Prep Kit 3.0' and 'SMRTbell® Barcoded Adapter Plate 3.0' kits (Pacific Biosciences of California, Inc.).

2.4. Sequencing data processing of full lenght RNAs

This study employed the IsoSeq workflow (https://isoseq.how/) to analyze bundled long-read RNA data from different samples. The sequencing data processing pipeline consisted of

several key steps: (1) data pre-processing using the pacbio software suite (PacBio. SMRT Link (v13.1) to select polyadenylated sequences, (2) alignment and transcript identification using the refrence genome GRCh38.p14 , (3) transcript refinement and relishing with the IsoSeq pipeline's Pigeon; this step is crucial for correcting errors and achieving high-quality isoform sequences, (4) characterization and classification of the isoforms.

3. Results

3.1 DNMT3B and telomerase activity significantly decrease in senescent keratinocytes

Our findings demonstrate a significant reduction of 34% in the production of DNMT3B in prematurely aged keratinocytes undergoing replicative senescence, compared to normal keratinocytes (Figure 2A). Additionally, the model exhibits a significant reduction in telomerase activity, which is typical of senescent cells (Figure 2B). These results provide initial evidence that our model effectively mimics conditions associated with cryptic transcription.

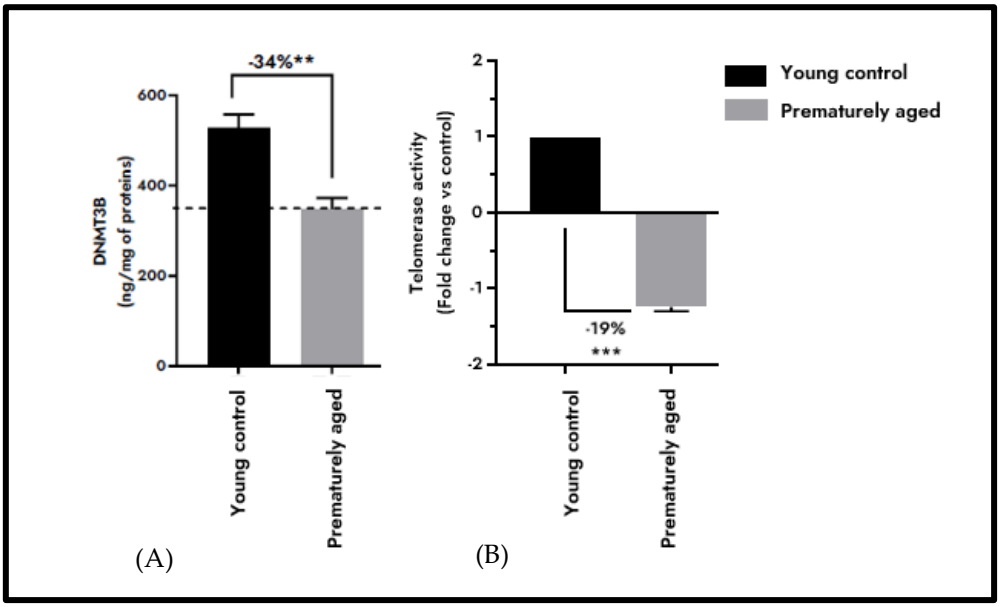

Figure 2. Quantification of DNMT3B expression expression (A) and telomerase activity (B) in keratinocytes. Activity is expressed in fold change vs control. Statistical analysis with Mann Whitney test and Student t test with #p<0.1, * p<0.05 and *** p<0.001.

3.2 Senescent keratinocytes exhibit a higher number of truncated transcripts

Full length RNA sequencing has enabled to identify truncated RNA. A higher number of Incomplete Splice Match (ISM) is detected in prematurely aged keratinocytes (+36%*) while full length RNA are similar between the two conditions, indicating that senescence contributes to an increase in frame shifts during transcript production (Figure 3).

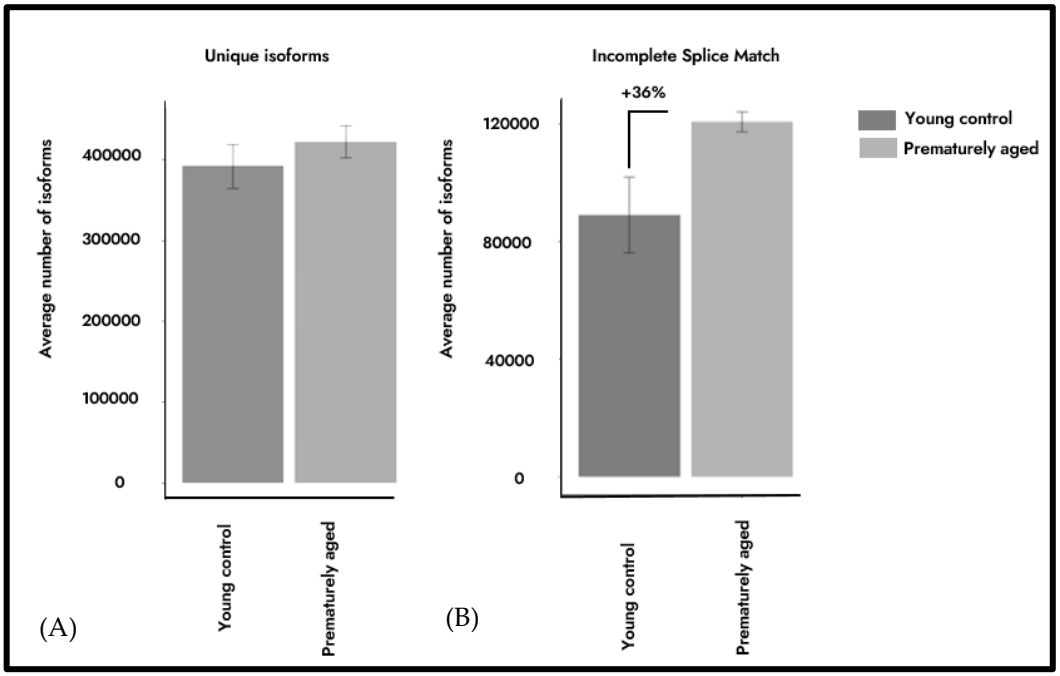

Figure 3. Prematurely aged keratinocytes present (A) a similar average number of unique isoforms and (B) a higher number of truncated RNA (Incomplete Splice Match, ISM). Average read counts of isoforms category for each condition of cells cultivation. Error bars represent +/- SD.

Specifically, we observed a higher prevalence of transcripts that were truncated in the 3' region (+44%), as well as transcripts that were truncated in both the 3' and 5' regions (+48%) or contained a single exon (+14%). Values are reported in Table 1. ISM 5’ fragment and ISM intron retention fragment were similar between the two conditions (Figure 4).

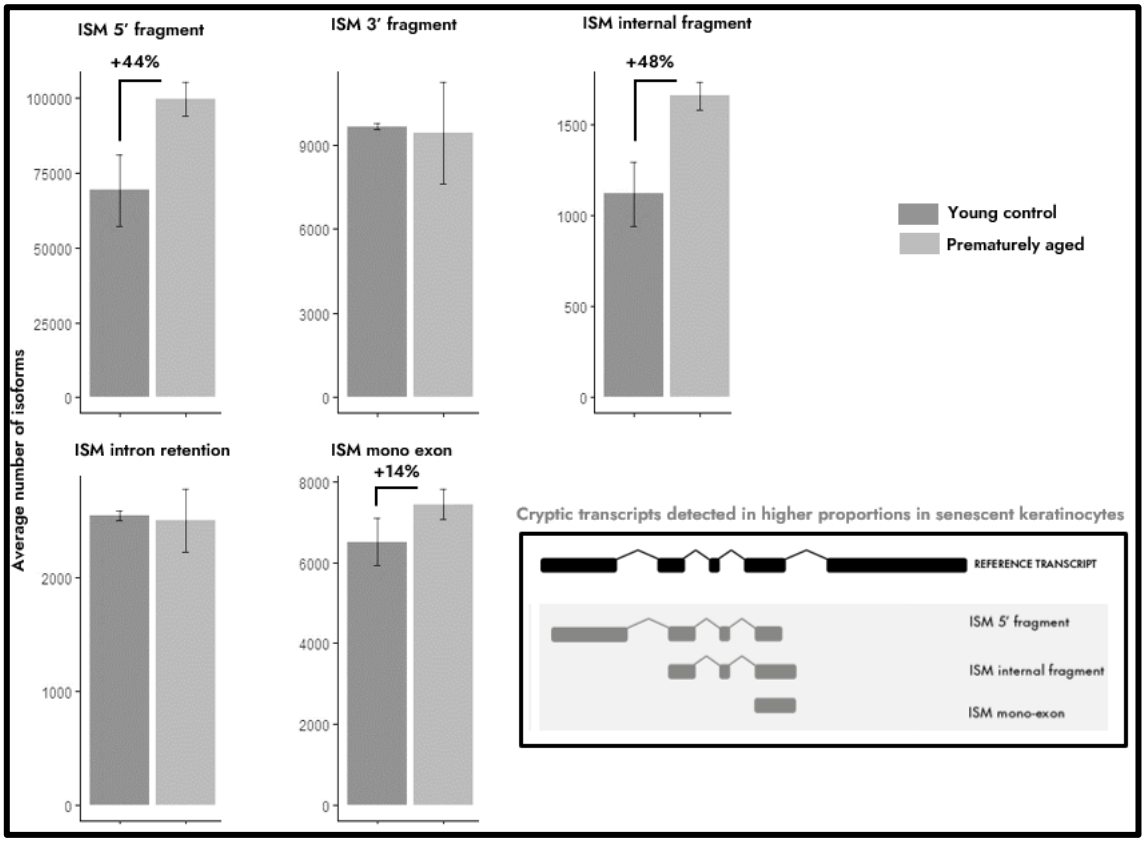

Figure 4. Prematurely aged keratinocytes present a higher number of ISM 5' fragments, ISM internal fragments and ISM mono exons. Average read counts per isoforms category for each condition of cells cultivation. Error bars represent +/- SD.

4. Discussion

The findings revealed a significant reduction of 34% in DNMT3B production and significant reduction of telomerase activity by 19% in prematurely aged keratinocytes compared to normal keratinocytes, indicating that the model effectively simulates conditions associated with cryptic transcription.

The sequencing study identified a 36% increase in RNA isoforms in prematurely aged keratinocytes, with higher proportions of truncated transcipts. Notably, there was a 44% increase in 3' truncated transcripts, a 48% increase in transcripts truncated at both ends, and a 14% increase in single-exon transcripts. These results suggest that transcriptional fidelity is dysregulated in prematurely aged keratinocytes and are summarized in figure 5 below.

Interestingly, ISM 5’ fragment and ISM intron retention fragment were similar between the two conditions. A complementary sequencing including a RNA selection by immunoprecipitation of 5’ caps could be useful to elimite a technical bias for the detection of ISM 5’ fragment.

Moreover, new investigations into the recognition of cryptic transcripts by the proteasome and the impact of cryptic transcripts on cell differentiation would be valuable for enhancing our understanding of this aging phenomenon.

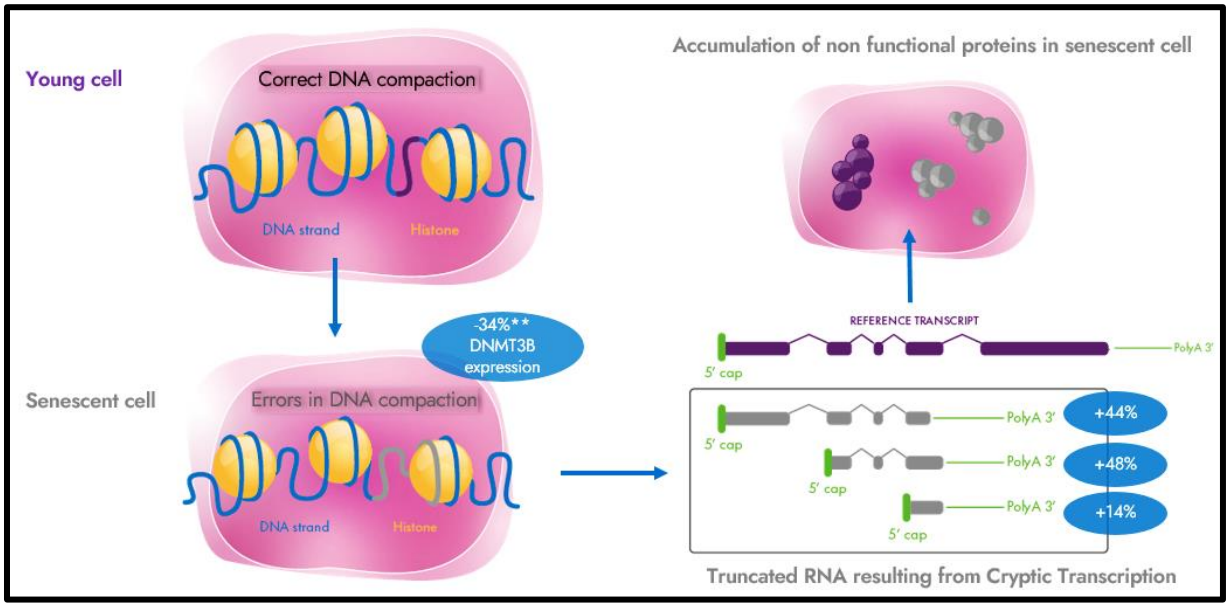

Figure 5. Visual abstract of the main findings related to cryptic transcription in senescent keratinocytes.

5. Conclusion

This study demonstrates the feasibility of studying cryptic transcription in skin cells through the analysis of full-length transcripts in a prematurely aged keratinocyte model. It contributes to the growing body of knowledge on the intricate relationship between transcriptional fidelity, senescence, epigenetics, and skin aging.

This transcriptomic world premiere paves the way for the development of cosmetic ingredients that target cryptic transcription and epigenetics to effectively control skin senescence, restore cellular functions, and promote skin longevity.

6. References

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