Effect of Millimeter Waves on Genome Architecture and the Transcriptome of Primary Human Fibroblasts

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***Abstract*—Millimeter waves (MMWs) are known to induce a range of biological effects, although the causes remain largely unknown. We exposed primary human fibroblasts to MMWs and observed changes in the genome architecture, with modified DNA secondary structure formation effecting gene expression and subsequent protein production. This work establishes the potential for MMWs to induce targeted modifications to the chromatin landscape for therapeutic applications.**

I. INTRODUCTION

HUMAN exposure to millimeter waves (MMWs) has historically been minimal due to strong attenuation by atmospheric oxygen molecules, meaning the current understanding of the biological effects of these frequencies is limited. Despite being non-ionizing, it is important to consider the biological effects of these frequencies from the context of increased natural exposure due to the adoption of MMWs for 5G mobile networking technologies and also as potential medical therapeutics, having been adopted for medical applications in several Eastern European nations [1].

Previous research has shown that MMWs can cause a range of biological effects at the cellular level, including modifications to biological membrane properties [2], gene expression [3] and altered neuronal action potentials [4]. However, the mechanisms causing these effects remain largely unknown. Initially attributed to the effect of heat shock, it has been shown that the changes in gene expression are not solely due to the thermal response [5], suggesting they arise from unique interactions with the genome.

G-quadruplexes (G4) and i-motifs (iM) are sequence specific non-canonical DNA secondary structures occurring within certain G- and C-rich regions of the human genome, respectively. Both structures are over-represented in regulatory regions such as gene promoters or telomeres, with implications in the regulation of transcription and genomic instability [6]. Many of the proposed interactions mechanisms of MMWs with DNA describe conditions that may alter the formation and stability of these structures, such as the induction of resonances causing transient single strandedness on the order of tens to hundreds of basepairs, known as DNA breathing modes [7], [8].

In this work primary human fibroblasts were exposed to 60 GHz MMWs with an average power density of 2.6 mW/cm^2. The cells were cultured in 4 wells of a 6-well plate, which was scanned through the 100 mW MMW beam continuously for 5 hours/day for 2-4 days. Characterization techniques including immunocytochemistry and targeted quantitative polymerase chain reaction have revealed MMWs stimulate the cells, modifying the genomic architecture and transcriptome.

II. RESULTS

We have observed modifications to the genome and transcriptome of primary human fibroblasts as a result of MMW exposure. Differential gene expression and subsequent protein production has been observed, while the formation and stability of DNA secondary structures is simultaneously affected.

Exposure to MMWs over 4 days induced increased deposition and coherency of extracellular collagen by the fibroblasts (Fig. 1.). Significantly more collagen was deposited per cell and the collagen fibers are more coherent, representing a greater alignment, key markers of fibroblast stimulation.

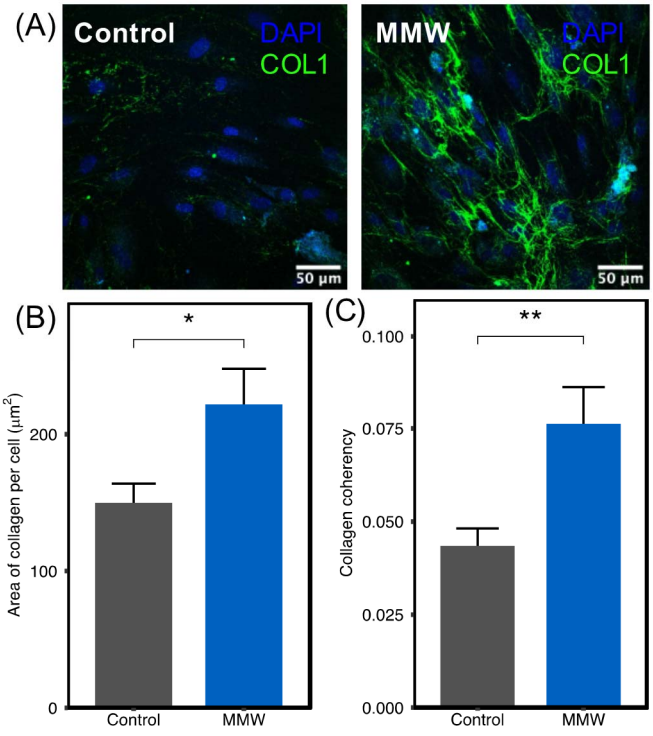

Fig 1. (A) Immunocytochemistry with COL1 antibody (green) reveals (B) that the production of extracellular collagen increases in fibroblasts exposed to MMWs over 4 days, corresponding to a significant increase in the area of collagen deposited per cell. (C) The deposited collagen fibers are more coherent for MMW exposed cells, representing a greater alignment, a key marker of fibroblast stimulation.

MMW exposure also results in time-dependent alterations to the transcriptome. After 2 days of 5 hours/day exposure, the expression of the COL1A1 gene, involved in the regulation of extracellular collagen production and deposition, increases 1.8-fold (Fig. 2.). This causes increased collagen production by the cells, resulting in the enhanced collagen deposition and coherency identified by immunocytochemistry.

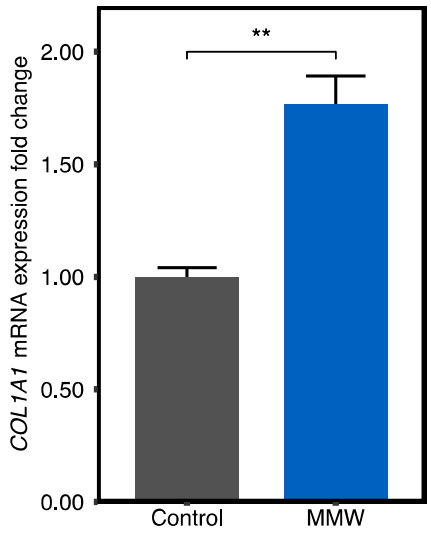

Fig 2. (A) Expression of the COL1A1 gene in primary human fibroblasts increases 1.8-fold due to MMW exposure for 5 hours/day over 2 days.

With many of the proposed interaction mechanisms of MMWs with DNA to modify gene expression including the induction of temporary single-strandedness via mechanisms such as DNA breathing modes or interactions with hydrogen bonds, it is possible that MMWs effect the formation of DNA secondary structures. The promoter region of the COL1A1 gene, for example, contains 3 regions with the potential to form G4s under physiological conditions, which may explain the modified expression of this gene. Visualization of both G4s and iMs via immunocytochemistry using the structure specific BG4 and iMab antibodies reveals the formation and stability of both structures are simultaneously affected (Fig. 3.).

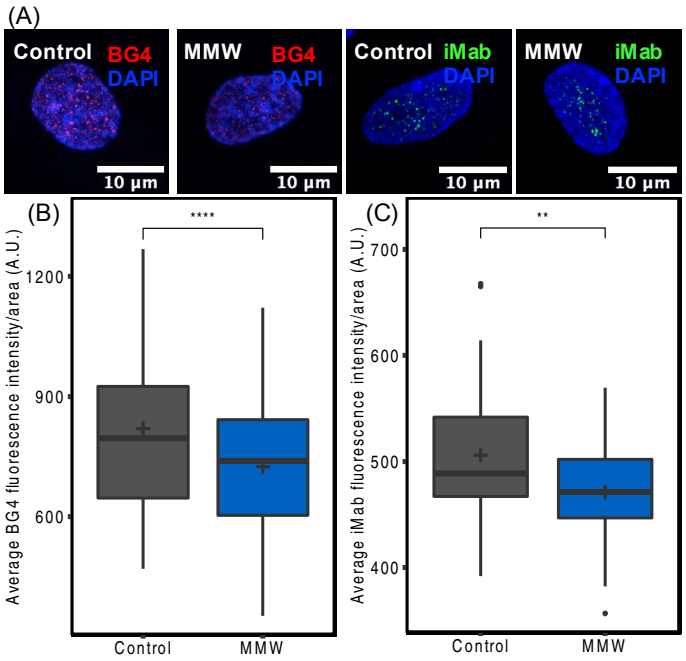

Fig 3. (A) Immunocytochemistry using structure specific BG4 (red) and iMab (green) antibodies allows visualization of G-quadruplex and i-motif populations in the nuclei of cells exposed to MMWs over 4 days. A decrease in the average fluorescence intensity of both the (B) BG4 and (C) iMab stained nuclei reveals that MMWs alter the formation and stability of both G4s and iMs simultaneously.

The simultaneous modification of both G4 and iM populations indicates that that MMWs directly interact with DNA to induce modifications to the genomic architecture. Modified formation and stability of these DNA secondary structures are known to impact the transcriptional machinery, with G4s tending to inhibit, and iMs promote, transcription of nearby genes [9]. This leads to changes in gene expression, in this case including upregulation of COL1A1, which in turn has downstream effects on protein production and biological processes. Thus, the direct modulation of the chromatin architecture by MMWs causes numerous cascading effects.

Negligible temperature changes occurred during exposure, suggesting these effects are not solely due to the thermal response.

III. Summary

We have demonstrated that MMWs interact with primary human fibroblasts, modifying the genomic architecture and resulting in altered gene expression and protein production. Modifications to the populations of G-quadruplex and i-motif structures reveals an interaction mechanism that may contribute to the altered transcriptome, resulting in numerous down-stream effects.

This describes a direct effect of MMWs on biological systems and establishes a mechanism whereby MMWs may be used to induce targeted modifications to the genome and transcriptome for therapeutic application.

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