ANALYSIS OF QUANTUM MOLECULAR RESONANCE EFFECTS ON HUMAN MESENCHYMAL STROMAL CELLS

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Background and aim

Endogenous electric fields play an essential role in cellular functions such as proliferation, migration and gene expression. Quantum Molecular Resonance (QMR) produces waves with a specific form at high frequencies and low intensity through electric fields without increase of temperature. Few is known about QMR mechanism of action on the inter/intracellular processes.

This work aims to evaluate as QMR acts on bone marrow derived mesenchymal stromal cells (BM-MSC).

Materials and Methods

BM-MSC, were treated with QMR (device supplied and patented by Telea, Italy) for 10 minutes for 4 consecutive days a week for 2 weeks (Figure 1). Cell morphology, phenotype, viability, proliferation and migration were investigated.

QMR effects on BM-MSC after the first week of stimulation were further investigated by microarray. For the latter, samples were processed according to the "Agilent Gene Expression Analysis". Data were analyzed by using the Limma package (R language). Differentially expressed genes between conditions were selected based on a p-value cut-off of 0.05. Gene enrichment analysis was performed using ToppGene Suite and Ingenuity Pathway Analysis tools.

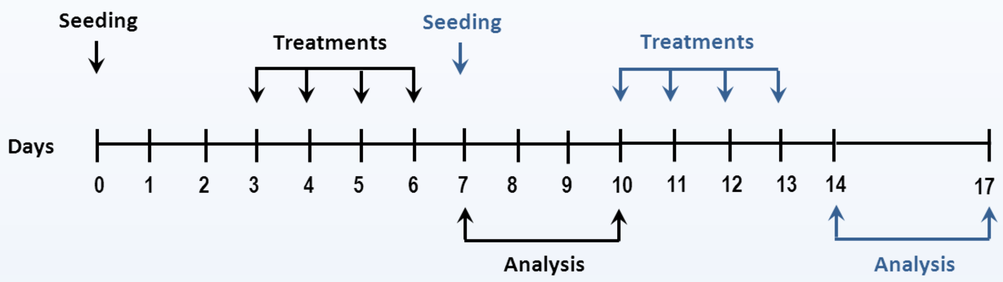

Figure 1. Scheme of QMR treatments. Cells were seeded on day 0, harvested and reseeded on day 7. On day 3 and day 10 the samples were either sham-exposed or treated with 40 or 80 nominal power for 10 minutes a day for 4 days. In black the 1st treatment cycle; in blue the 2nd treatment cycle.

Results

The observations related to morphology and phenotype (Figure 2 and 3, respectively) suggested the maintenance of BM-MSCs identity after 2 weeks of QMR treatment. Furthermore, no alteration of cellular viability, proliferation and migration were observed between samples and controls (Figure 4) .

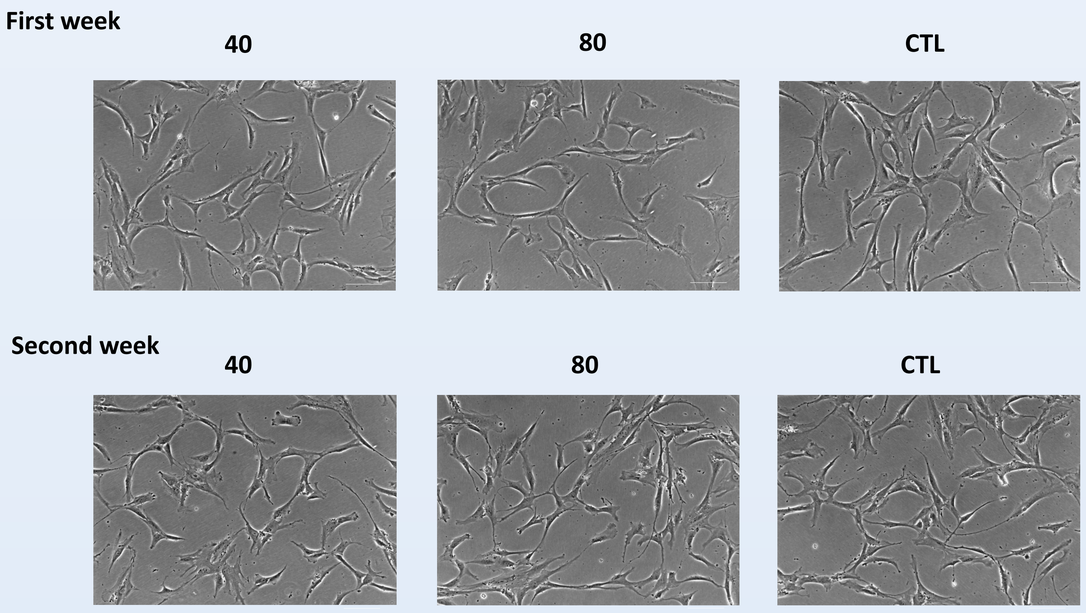

Figure 2. BM-MSCs morphology of a representative sample at Day5 (first week of treatment) and at Day12 (second week of treatment). Scale bar =100 μm. Total magnification = 100x .

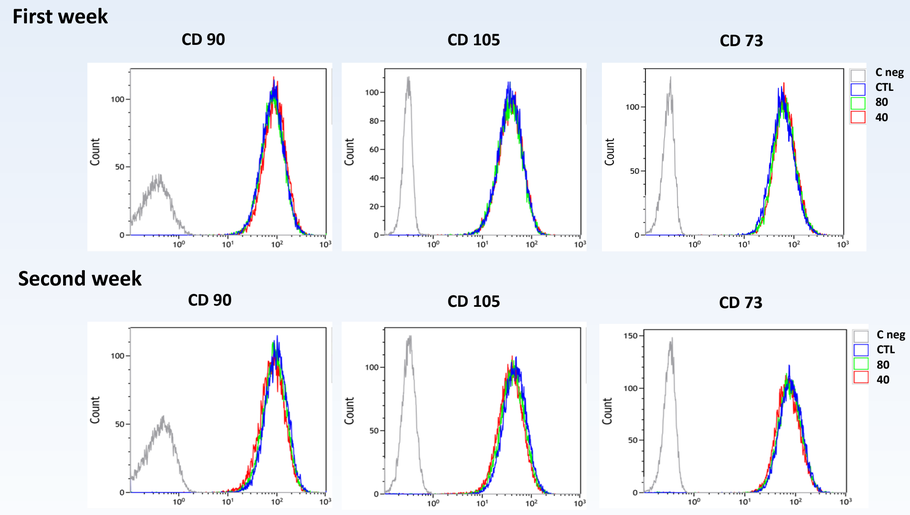

Figure 3. BM-MSCs phenotype of a representative sample after 1 and 2 cycles of QMR stimulation. Grey line=unstained control. Blue line=sham-exposed control. Green line=QMR setting 80. Red line=QMR setting 40.

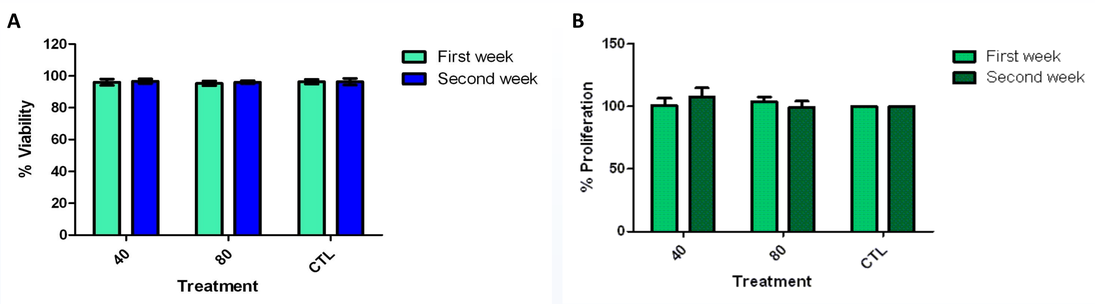

Figure 4. QMR effects on BM-MSCs viability and proliferation. A) Viability was determined by flow cytometry using LIVE/DEAD Fixable far red stain kit (Invitrogen). B) Cellular proliferation was determinated by WST-1 assay (Sigma-Aldrich). Data were expressed as % of proliferation VS control.

At molecular level, the QMR treatment seemed to slightly affect the cell transcriptome: the identified up-regulated genes were mainly involved in cell tissue and vasculature development while the down-regulated genes were involved in cellular growth, phosphorylation, movement and anchoring (Table 2).

Comparison	N° up-regulated genes	Fold-change (max)	N° down-regulated genes	Fold-change (max)	p-value	Adjusted p-value
40 treatment vs control	411	2,5	987	2,3	<0,05	<0,496
80 treatment vs control	163	2,9	199	1,4	<0,05	<0,999

Table 1. Differentially expressed genes between treated BM-MSC cultures and sham-exposed controls.

Category	N° lists	Changed genes	Fold-change ≥ 1,3
40 up-regulation	***13***	***23***	***18***
Cellular Development	2	16	11
Tissue Development	3	12	9
Cell Differentiation	2	6	5
Cardiovascular Development	6	10	8
40 down-regulation	***7***	***58***	***50***
Phosphorylation	3	16	14
Cellular Development	2	41	38
Cellular Migration	1	27	26
Anchoring junction	1	5	5
80 up-regulation	***4***	***3***	***3***
Extracellular Matrix Organization	2	2	2
Cellular Development	2	1	1

Table 2. Best enrichment gene lists considering outputs of the software ToppGene Suite and Ingenuity Pathway Analysis (q-value FDR B&H<0.01 and B-H p-value<0.01, respectively). A p-value≤0.005 for the changed genes was considered statistically significant. At this level of analysis, the 80 down-regulation condition doesn't show significant genes.

Conclusions

The QMR treatment maintained the BM-MSCs identity and growth and no alteration of the cell phenotype were observed. The microarray analysis evidenced weak transcriptional differences between conditions. Nevertheless, the gene enrichment in tissue and vasculature development processes might suggest that QMR could activate angiogenesis. This hypothesis requires further investigation.