
ASSESSMENT OF SELECTED MOLECULAR FACTORS IN CORRELATION WITH DISEASE PROGRESSION AND EFFECTIVENESS OF HSCs CD34+ TREATMENT IN PATIENTS WITH KNEE OSTEOARTHRITIS

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Hypothesis: Intra-articular injection of CD34+ cells, in the knee joint of patients with OA, modulates the expression levels of anabolic and catabolic markers, influencing articular cartilage homeostasis process.

OBJECTIVE OF THE WORK

Assessment of the effect of treatment with intra-articular CD34+ cells injection in relation to the selected molecular anabolic/catabolic markers characteristic for knee osteoarthritis development.

Specific goals

Assessment of the gene expression level: *FGF2*, *HIF-2 α* , *ADAMTS-4*, *ACAN* and miRNA: miR-16 and miR-335 in joint aspirate collected from patients diagnosed with OA:

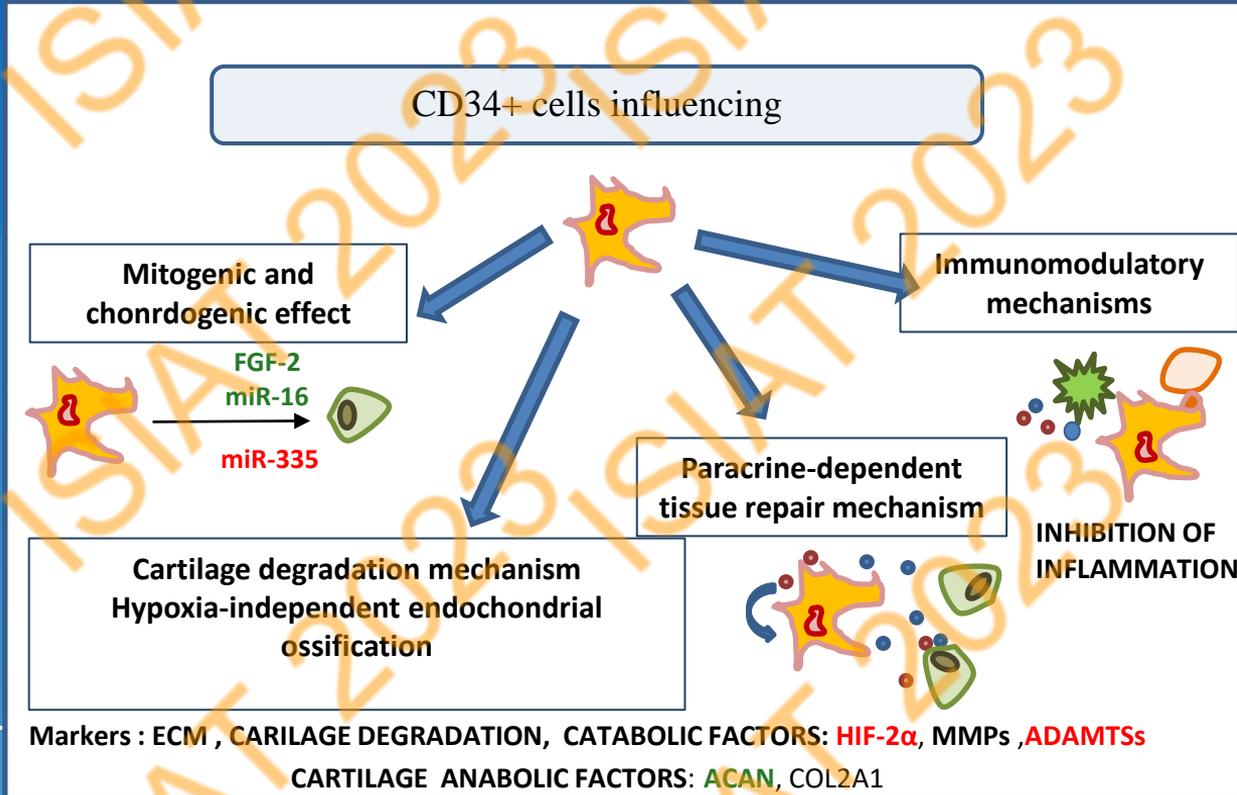
- Before the intra-articular injection of CD34+ cells (T0) and one year after (T1),
- Correlation with the OA stage and the total number of CD34+ cells injected

OSTEOARTHRITIS (OA)

- is an incurable joint disease manifesting itself with gradually progressing degenerative changes, leading to premature motor disability. These changes mainly occur owing to an imbalance between the processes of degeneration and regeneration of articular cartilage structures.

Therapeutic strategies in the treatment of OA:

1. Pharmacological treatment
2. Surgical interventions
3. Autologous chondrocyte implantation, autologous conditioned serum (ACS) injection
4. Orthobiological treatment with platelet-rich plasma (PRP) - platelet derived growth factor (PDGF)
5. Biological treatment - tissue inhibitors of metalloproteinases (TIMPs)



Biological material

Knee joint aspirate from patients diagnosed with OA

Time point T0
(before intra-articular
injection of CD34+ cells)
N=54

Time point T1
(12 months after
injection of CD34+ cells)
N=54

**Apheresis-derived CD34+ cells
assessed by flow cytometry were
administered as a single intra-
articular injection**

**Quantification of CD34+ cells:
 $1.8 \times 10^5 - 1.25 \times 10^6$
(average cells count: 801 978)**

30% of patients diagnosed with osteoarthritis experience difficulty walking, and approximately 80% of patients experience knee joint pain

Table 1. Clinical characteristics of patients diagnosed with OA

	Number of patients (n)	Percentage of patients (%)
Sex		
women	32	59
men	22	41
Age		
≤ 60	35	65
> 60	19	35
Stage of OA (Outerbridge classification)		
I+II	3	5,5
III	16	30,5
IV	21	57

Methods

- Obtaining a fraction of CD34+ cells by apheresis from the peripheral blood of patients diagnosed with OA
- Cytometric evaluation of CD34+ cells
- Intra-articular injection of 0.9% NaCl (40 ml) to flush the joint and take an aspirate (**T0**)
- Injection of CD34+ cells obtained from the patient into the knee joint

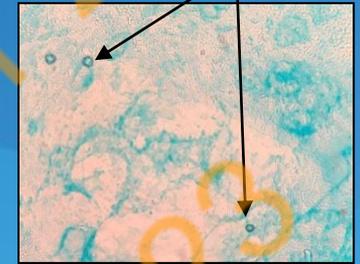
T0

- Intra-articular injection of 0.9% NaCl (40 ml) to flush the joint and collect an aspirate 12 months after administration of CD34+ cells to the knee joint (**T1**).

T1

- RNA isolation from the collected aspirate (mirVana miRNA Isolation Kit)
- Qualitative and quantitative assessment of the isolated RNA (Eppendorf BioPhotometer UV/VIS spectrophotometer)
- Analysis of the relative expression level of selected genes and miRNAs by qRT-PCR using TaqMan probes in the 7900 HT Fast Real-Time PCR device

Chondrocytes



Statistical analysis

(U-Mann Whitney, Kruskal-Willis, Wilcoxon)



Results (1)

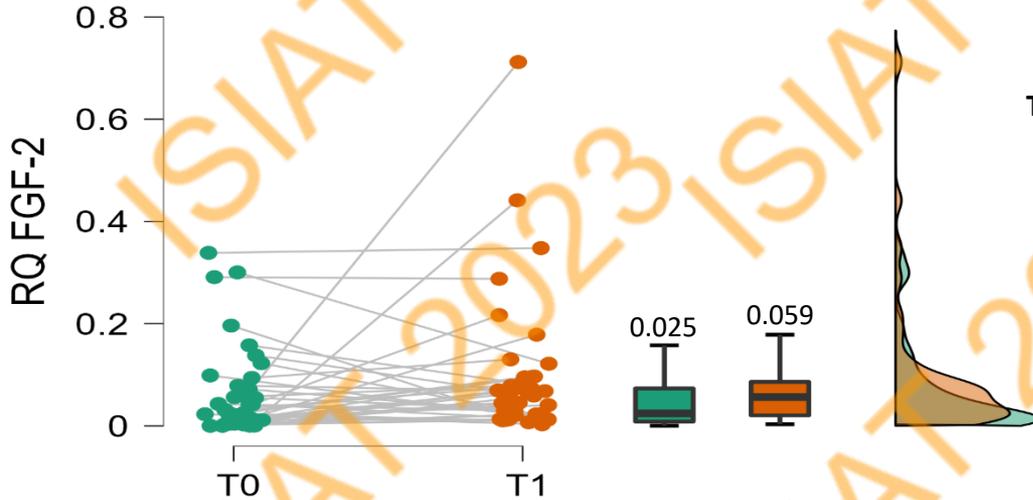


Table 2. Analysis of selected genes expression level (T0 vs. T1) for total group of patients with OA diagnosis

		<i>HIF-2α</i>	<i>ACAN</i>	<i>ADAMTS-4</i>	<i>FGF-2</i>
Median [RQ]	T0	0.0536	0.05295	0.0674	0.0247
	T1	0.0676	0.0009	0.069	0.0592
p		0.114	<0.001	0.772	0,548

Table 3. Analysis of OA stage impact on selected genes expression level (T0 vs. T1)

		<i>HIF-2α</i>				<i>ACAN</i>				<i>ADAMTS-4</i>				<i>FGF-2</i>			
OA stage		I+II	III	IV	p	I+II	III	IV	p	I+II	III	IV	p	I+II	III	IV	p
Median [RQ]	T0	0.091	0.063	0.053	0.975	0.091	0.096	0.038	0.530	0.120	0.088	0.039	0.421	0.096	0.039	0.019	0.235
	T1	0.142	0.102	0.076	0.827 (III vs.IV)	0.0001	0.003	0.0002	0.015 (III vs.IV)	0.018	0.133	0.070	0.211 (III vs.IV)	0.121	0.070	0.034	0.049 (III vs. IV)
p		-	0.393	0.614		-	0.036	0.065		-	0.494	0.339		-	0.070	0.386	

Wilcoxon test

Results (2)

Table 4. Analysis of selected miRNAs expression level (T0 vs. T1) for total group of patients with OA diagnosis

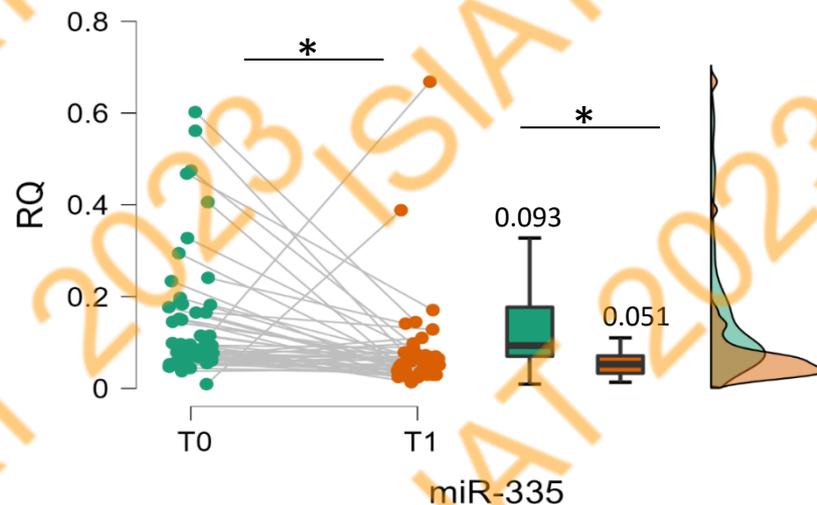
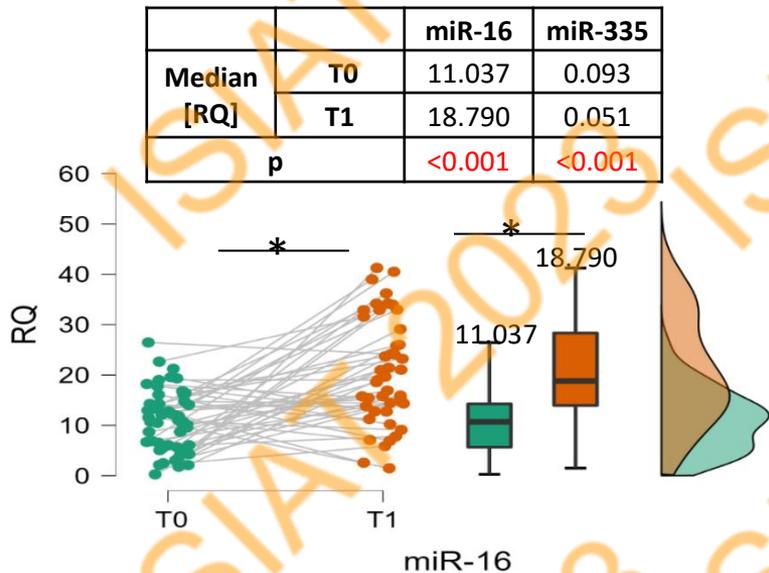


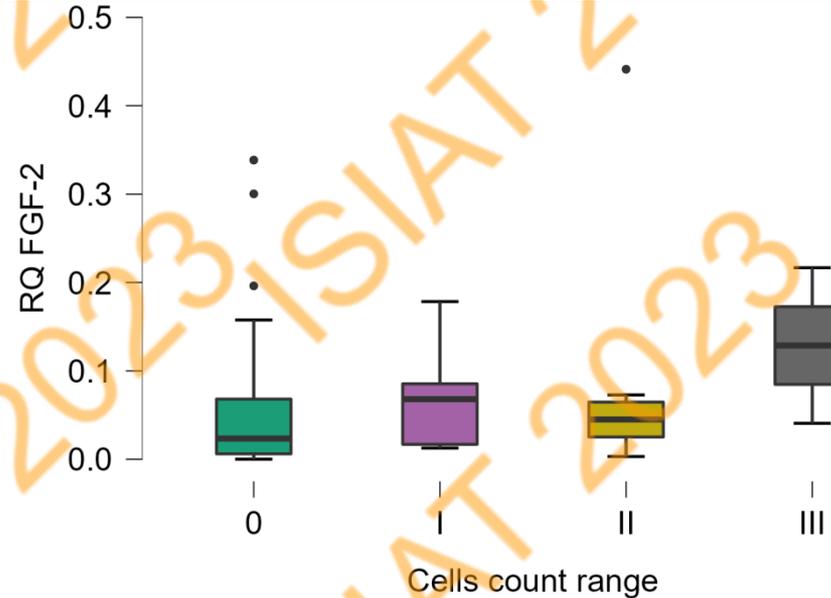
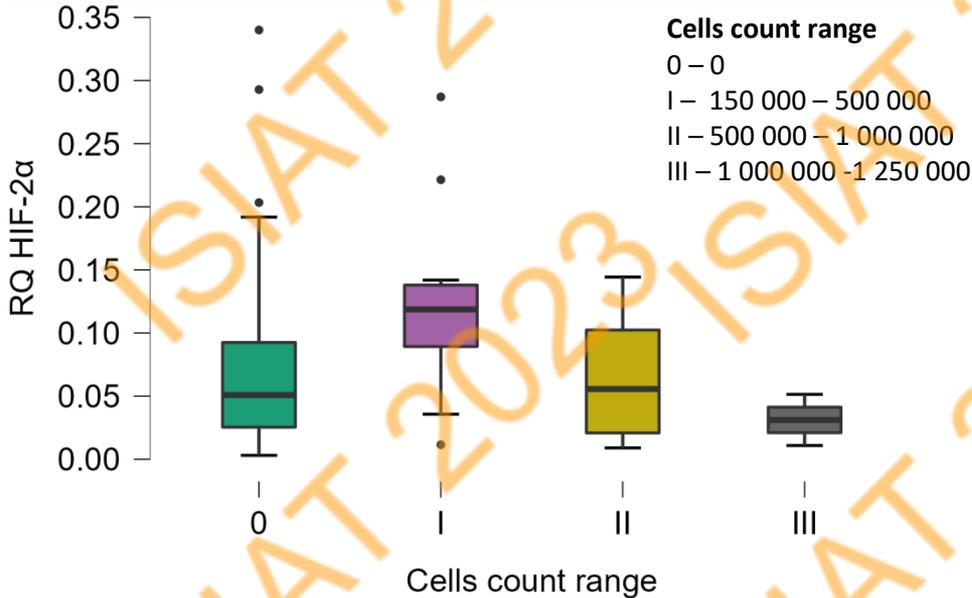
Table 3. Analysis of OA stage impact on selected miRNAs expression level (T0 vs. T1)

		miR-16				miR-335			
OA stage		I+II	III	IV	p	I+II	III	IV	p
Median [RQ]	T0	7.180	10.128	8.703	0.818	0.146	0.098	0.104	0.820
	T1	5.857	18.573	23.225	0.222	0.171	0.053	0.043	0.219
p		-	0.015	<0.001		-	0.513	0.045	

Results (3)

Table 6. Analysis of selected genes expression level according to injected CD34+ cells number

	HIF-2 α				ACAN				ADAMTS-4				FGF-2			
	T0	T1			T0	T1			T0	T1			T0	T1		
Cells count range	0	I	II	III	0	I	II	III	0	I	II	III	0	I	II	III
Median [RQ]	0.051	0.119	0.056	0.031	0.033	0.0003	0.001	0.004	0.069	0.052	0.133	0.254	0.023	0.068	0.045	0.129
p	0.096				<0.001				0.552				0.376			

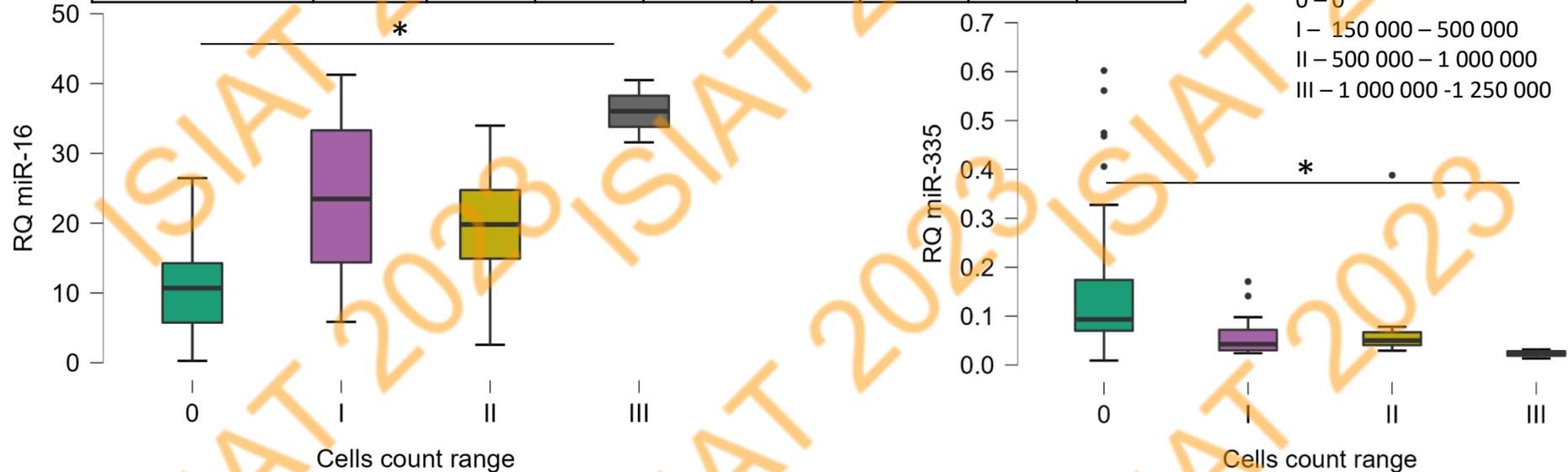


Results (4)

Table 7. Analysis of selected miRNAs expression level according to injected CD34+ cells number

	miR-16				miR-335			
	T0	T1			T0	T1		
Number of cells	0	I	II	III	0	I	II	III
Median [RQ]	10.700	23.465	19.805	36.029	0.093	0.043	0.050	0.025
p		0.0001				0.00030		
		0.0015	0.0035	0.0006		0.0011	0.0314	0.0006

Cells count range
 0 – 0
 I – 150 000 – 500 000
 II – 500 000 – 1 000 000
 III – 1 000 000 -1 250 000



Summary

- 1) The effect of CD34+ cells on miRNA expression level is observed after injection of smaller amount of cells (above 500 000) compared to effect on gene expression (above 1 000 000).
- 2) CD34+ cells injection influenced the increase in expression level of **ADAMTS-4**, **HIF-2 α** , **FGF-2** ($p>0.05$) and decrease in expression level of **ACAN** ($p<0.05$).
- 3) CD34+ cells injection influenced the increase expression level of **mi-16** and decrease expression level of **miRNA-335** at T1 compared to T0 ($p<0.05$).

Conclusion

The effect of the injected CD34+ cells on OA chondrocytes depending on the amount of cells. Injection of CD34+ cells didn't reduce expression of matrix catabolic study markers in joint cartilage and proanabolic effect was not observed. At this stage of the study, the positive effect of CD34+ cells on the articular cartilage maintaining homeostasis process was not unequivocal confirmed.

Thank you for your attention

