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Expression of the *BIRC5* gene in the presence of adalimumab in normal human dermal fibroblasts (NHDF)

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ABSTRACT

Survivin encoded by *BIRC5* belongs to the group of proteins that inhibit apoptosis. It consists of the BIR and α -helical C domains. In addition to its inhibitory activity, it plays an important role in cell cycle regulation. Adalimumab is an immunosuppressive drug, a recombinant human anti-TNF- α monoclonal antibody. It is used in the treatment of autoimmune diseases. The aim of the study was to evaluate changes in the expression of *BIRC5* and genes encoding apoptosis inhibitors (IAP), depending on the exposure time of the cells to adalimumab. The study material consisted of normal human dermal fibroblasts (NHDF) cultured under standard conditions in the presence of adalimumab (8 μ g/mL) for 2, 8 and 24 hours. The expression profile of genes associated with apoptosis was determined with the use of HG-U133A 2.0 oligonucleotide microarrays (Affymetrix). The comparative analysis was performed with one-way ANOVA and Tukey's HSD tests ($p < 0.05$) using the PL-Grid Infrastructure (<http://www.plgrid.pl/en>). In this study, it was determined that the number of mRNAs encoding proteins from the IAP family, differentiating the NHDF culture exposed to the anti-TNF drug from the control, varies depending on the exposure time of the cells to the drug (2h, 8h, 24h) and is as follows: 2h, 5 mRNAs; 8h, 6 mRNAs; 24h, 1 mRNAs. 1 ID mRNA changes its transcriptional activity regardless of the exposure time of cells to the drug and it is *BIRC5* ($p < 0.05$). The FC value for this mRNA is as follows: 2h vs. C, -1.59; 8h vs. C, -1.38; and 24h vs. C, 1.16. The effect of adalimumab on the transcriptional activity of the gene encoding survivin depends on the exposure time of the cells to the

drug. *BIRC5* is a gene differentiating the culture of normal human dermal fibroblasts regardless of the exposure time to adalimumab. The direction of expression change depends on the time of exposure to the drug.

Keywords: survivin, adalimumab, NHDF

1. INTRODUCTION

One of the greatest achievements of modern medicine is the introduction of biological medicines for the treatment of autoimmune diseases. This type of therapy carries with it a good chance of improving the life quality of people suffering from high severity of those diseases, including psoriasis. In order to learn about the immunopathogenesis of this disease, studies on the human genome has been conducted over the past several years. Thus obtained information and reports are an important reference point for scientists in the context of the application of new biological drugs. [1-3]. These medicines, compared to other therapeutic methods, are relatively new, therefore their long-term effects are not fully understood. One of the drugs in this group is adalimumab. It is a fully human monoclonal antibody that binds to tumor necrosis factor (TNF- α), resulting in inhibition/reduction of the inflammatory process due to the inactivation of signal cascades activated by TNF- α receptors. [4].

Inhibitor of apoptosis (IAP) proteins are a group of endogenous polypeptides present in mammals and involved in the regulation of apoptosis. There are eight proteins among this group: XIAP (Human X Chromosome-Encoded IAP), ML-IAP/Livin (Melanoma IAP), IAP-1 (Cellular IAP-1), IAP-2, ILP-2 (IAP-like Protein 2), BRUCE/Apollon, NAIP (Neuronal Apoptosis-Inhibitory Protein) and survivin [5]. The structure of all IAP proteins is based on the presence of at least one BIR (Baculoviral IAP Repeat) domain. In addition, there are specific domains for each of the proteins. The BIR domains, as functional units of IAP proteins, ensure the specific action of individual proteins. The BIR domain takes the form of globular structures, which consists of 3 to 5 α -helices and several β -sheets. Through these domains, it is possible to combine proteins with caspases, causing their inactivation [6].

BIRC5 is a gene encoding survivin, belonging to the group of proteins that inhibit apoptosis. Survivin was discovered by Ambrosini et al. in 1997 [7]. Its biological significance is mainly based on ensuring the continuity of cell divisions, and therefore its absence causes cell death through apoptosis [8-10]. A single BIR domain of survivin has a broad blocking effect on caspase-3, -7 and -9 [10-12].

Survivin is a unique protein due to its exclusive expression in normal proliferating tissues and in cancer cells. This phenomenon is not observed in cells of normal non-proliferating tissues. Therefore, survivin was referred to as a potential diagnostic marker and is the target of gene therapy, including cancer. [13, 14].

AIM

The aim of this study conducted on the culture of normal human dermal fibroblasts (NHDF) treated with adalimumab was to assess the changes in the transcriptional activity of the gene encoding survivin, in relation to other genes encoding IAP proteins depending on the exposure time of cells to the biological drug adalimumab.

2. EXPERIMENTAL

2. 1. Material and Methods

In the first stage of the study, normal human dermal fibroblasts (CC-2511 Lonza, Basel, Switzerland) were cultured under standard conditions: 5% CO₂, 37 °C, 90% humidity provided by Direct Heat CO₂ Incubator (Thermo Scientific, Waltham, MA, USA). The cultures were maintained in Fibroblast Basal Medium (FBM; Lonza, Basel, Switzerland) supplemented with Human Fibroblast Growth Factor-basic (hFGF-b), gentamicin and insulin (FGMTM SingleQuots™; Lonza, Basel, Switzerland). Cells with a viability ≥ 98% were used in the experiment. After reaching the subconfluent state (70%), adalimumab was added at a concentration of 8.00 μL/mL medium. The cells were incubated with the drug for 2, 8 and 24 hours. The control for the experiment was untreated normal human dermal fibroblasts. Total RNA was extracted for molecular analysis from NHDF cells with the use of TRIzol® reagent (Invitrogen Life Technologies, California, USA) and then purified with RNase-Free Dnase Set Mini Kit (Qiagen GmbH, Germany). The expression profile of genes associated with apoptosis was assessed using GeneChip® Human Genome U133A 2.0 arrays (Affymetrix, Inc. California, USA). Amplified RNA (aRNA) obtained by labeling RNA with biotin has been hybridized with probes on HG-U133A microarrays. The hybridization mixture was prepared with the GeneChip, Hybridization, Wash and Stain Kit (Affymetrix, Santa Clara, CA). The Affymetrix GeneArray Scanner 3000 7G as well as the GeneChip® Command Console® Software (Affymetrix, Santa Clara, CA) were used to assess the fluorescence intensity of the aRNA. The next step was to identify and select genes differentiating normal human dermal fibroblasts exposed to the biological drug from the control using Affymetrix NetAffx™ Analysis Center database (<http://www.affymetrix.com/analysis/index.affx>). Comparative analysis was performed with the use of the PL-Grid Infrastructure (<http://www.plgrid.pl/en>).

In addition, one-way ANOVA and post-hoc Tukey's tests were performed.

2. 2. Result

The first step of results analysis included the assessment of differences in the mRNA fluorescence profile in normal human dermal fibroblasts exposed to adalimumab and in control cells after 2, 8 and 24 hours. For this purpose, hierarchical cluster analysis with Euclidean distance was performed on the expression profile of 10 mRNAs corresponding to apoptosis inhibitor proteins (IAPs). It was observed that the change in expression after 24 hours is similar to that observed in the control while changes after 2 and 8 hours are similar to each other. The number of mRNAs differentiating study groups from the control was as follows: 2h vs. C, 5; 8h vs. C, 6; 24 vs. C, 1 (Fig. 1, Tab. 2, Fig. 2).

Table 1. The number of mRNAs associated with IAP proteins differentiating cells exposed to the drug from the control determined by a one-way ANOVA.

p-value		p < 0.05	p < 0.02	p < 0.01	p < 0.0050	p < 0.0010
mRNAs	10	7	7	7	6	5

Table 2. The number of mRNAs associated with the apoptosis pathway differentiating NHDF cells exposed to the anti-TNF drug from the control (p <0.05)

	The number of mRNAs			
	[C]	[24h]	[2h]	[8h]
[C]	7	1	5	6
[24h]	6	7	5	5
[2h]	2	2	7	4
[8h]	1	2	3	7

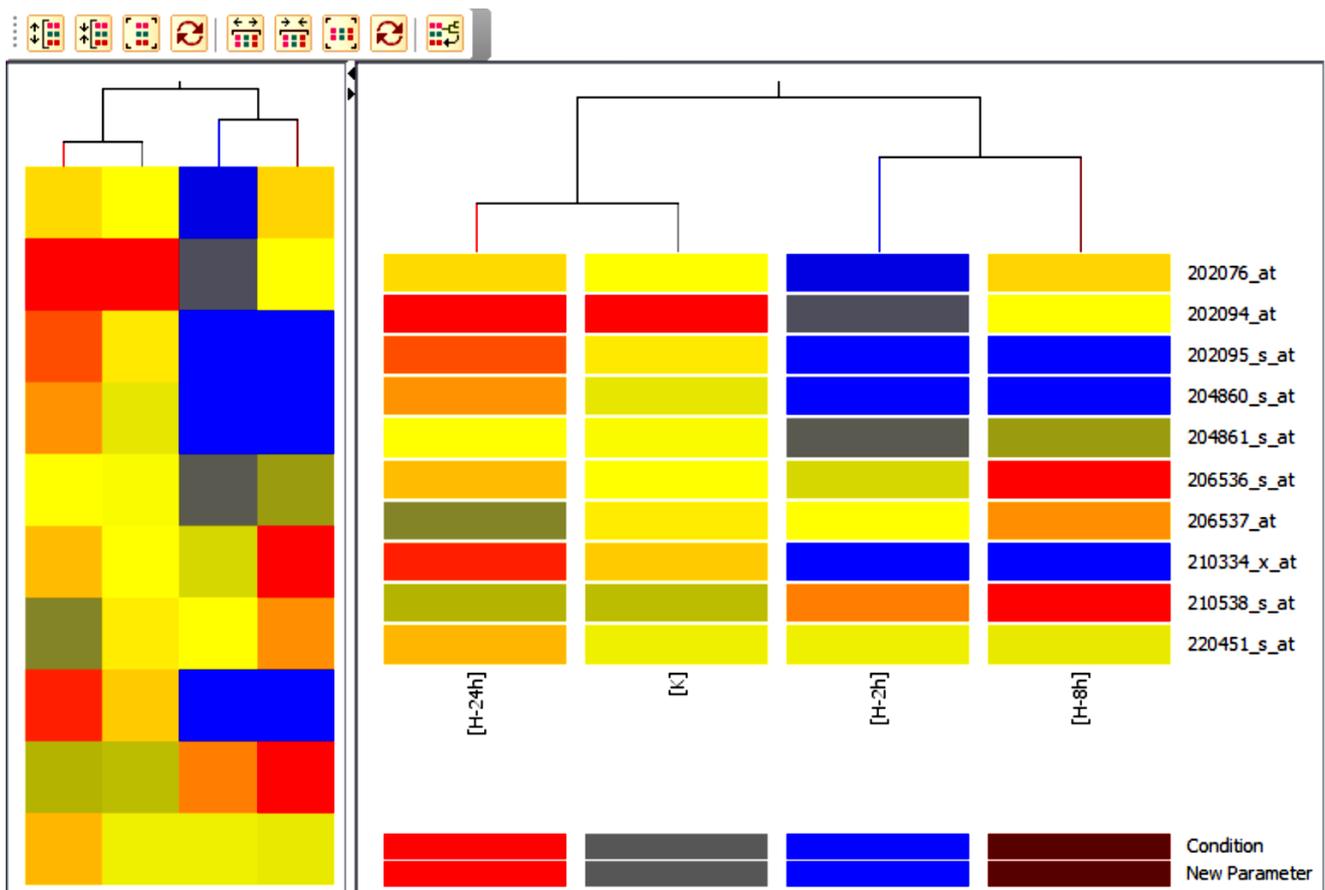


Figure 1. Hierarchical clustering of transcriptomes (10 mRNAs associated with IAP proteins) of dermal fibroblasts depending on the exposure time of cells to adalimumab.

The next stage of the comparative analysis consisted of performing one-way ANOVA and Tukey's post-hoc tests in order to identify which of the analyzed mRNAs differentiate study groups regardless of the exposure time of fibroblasts to the biological drug. The obtained results indicate that among 10 mRNAs associated with IAP family proteins, 7 differentiate the examined cells at $p < 0.05$ (Table 1). The next step was to determine the number of mRNAs that differentiate study groups at different exposure times (2h, 8h, 24h). Tukey's post hoc test was used, which showed that among 7 mRNAs selected in the ANOVA test, 5 mRNAs differentiate NHDF cells after 2 hours of adalimumab exposure from the control, 6 mRNAs after 8 hours and 1 mRNA after 24 hours (Tab.2).

Venn diagram was then generated, which allowed to observe that 1 mRNA changes its transcriptional activity regardless of the exposure time of fibroblasts to the biological drug at $p < 0.05$ (Fig. 2).

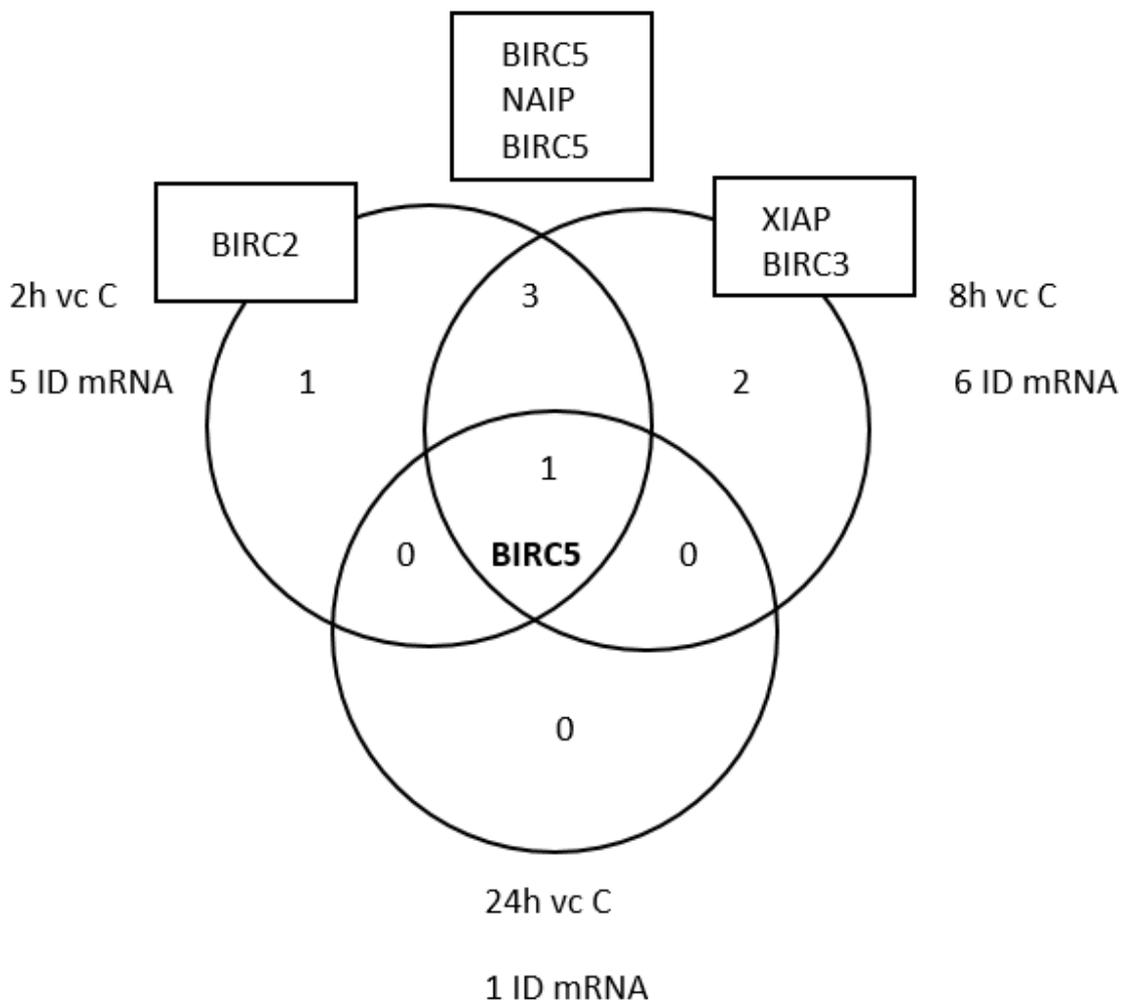


Figure 2. Venn diagram showing the number of specific mRNAs associated with IAP family proteins depending on the time of drug exposure - 2h, 8h, 24h ($p < 0.05$).

The gene that changed its transcriptional activity regardless of the exposure time of normal human dermal fibroblasts to adalimumab is *BIRC5* encoding survivin.

Analysis of the expression profile of the *BIRC5* gene shows that the biological drug (adalimumab) induces a reduction in its expression after 2 and 8 hours of drug exposure of NHDF cells compared to the control, while after 24 hours a relatively low overexpression of this gene is observed, comparable to that observed in the control culture (Table 3, Fig. 3).

Table 3. Result of the *BIRC5* expression analysis showing the effect of adalimumab depending on the exposure time with a focus on the direction of change when compared to the control.

Probe Set	Gene Symbol	p-value	Absolute FC 2h vs C		Absolute FC 8h vs C		Absolute FC 24h vs C	
			direction	value	direction	value	direction	value
202094_at	<i>BIRC5</i>	0.0000004	down	1.5935395	down	1.3847797	up	1.1585505

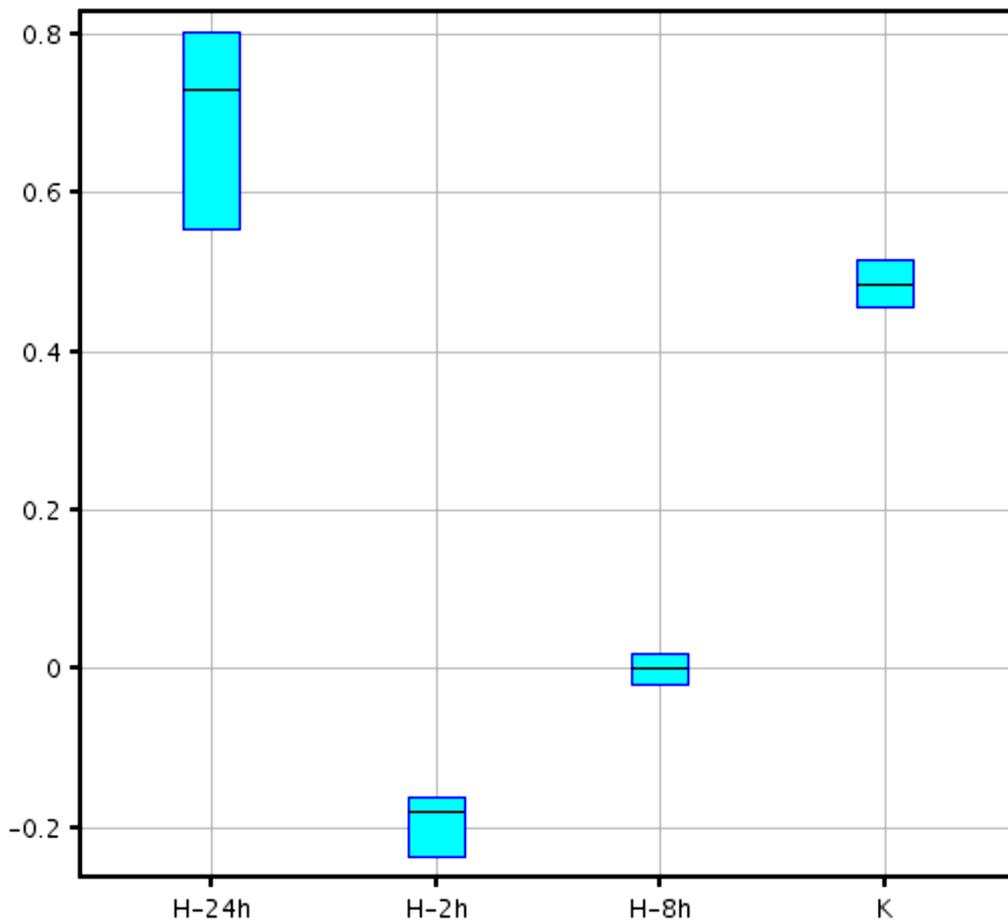


Figure 3. Box plot showing *BIRC5* expression depending on the exposure time to adalimumab (2h, 8h, 24h) [Probe Set - 202094_at].

3. CONCLUSIONS

Biological drugs are the newest therapeutic line in the treatment of severe forms of psoriasis. Satisfactory effects are also obtained in the case of joint symptoms of this disease.

Biological therapy is directed at the molecular aspect of a given disease, in this case psoriasis. One of the methods of therapy is the use of anti-TNF drugs, including adalimumab - a human anti-TNF monoclonal antibody [15].

Determination of molecular markers in the treatment with biological drugs brings the possibility to assess the effectiveness of the applied therapeutic procedure, and in the case of its failure - a change of strategy, and thus the choice of different biological medicine. Therapeutic failure may result from the emerging drug resistance to a particular medicine, manifesting as side effects [16].

In this study, the authors have attempted to evaluate the ability to modulate the transcriptional activity of *BIRC5* encoding survivin, a protein belonging to the IAP group, by adalimumab in the culture of normal human dermal fibroblasts. In addition to the inhibitory effect on apoptosis, this gene also participates in the regulation of the cell cycle [17].

The obtained results indicate that molecular changes are observed as early as 2 hours after administration of the drug, while the effects expected as a result of the application of biological medicine are observed after 24 hours. Overexpression of the gene encoding survivin prevents induced and spontaneous apoptosis. This proves the effectiveness of the used anti-TNF drug.

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