SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of STAT signaling pathways in human tumor cell lines using single-cell methods

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The Examination takes place at the library of Department of Physiology, Faculty of Medicine, University of Debrecen, March 16, 2023, 11:00 am.

Head of the **Defense Committee**:	László Csernoch, PhD, DSc
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The PhD Defense takes place at the F.003-004 Lecture Hall of Life Sciences Building, Faculty of Medicine, University of Debrecen, March 16, 2023, 1:00 pm.

INTRODUCTION

STAT proteins

STAT proteins, which belong to the family of cytoplasmic transcription factors, transmit signals to the nucleus by ligand binding to cell surface receptors. Seven STAT proteins encoded by different genes have been described in mammals, namely STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. Genes activated by STAT proteins play a critical role in regulating cell proliferation, survival and differentiation, as well as functional activity, and their involvement is essential in many physiological processes. STAT activation is transient and tightly regulated under physiological conditions, and imbalances in these mechanisms can lead to pathological processes, such as the development of malignant lesions.

Structure of STAT3

STAT3, similar to other STAT proteins, is composed of six highly conserved domains with distinct and identifiable functions starting from the N-terminal end, namely (1) N-terminal domain (NTD), (2) coiled-coil domain, (3) DNA-binding domain (DBD), (4) linker region (LR), (5) Src homology domain (SH2), (6) transactivation domain (TAD). The transactivation domain contains the two phosphorylation sites of STAT3 at the tyrosine residue at position 705 and the serine residue at position 727.

The IL-6/IL-6R/STAT3 signalling pathway

IL-6 is produced by many types of cells, including monocytes, macrophages, fibrocytes, activated T and B cells and some tumor cells. Increased IL-6 production and dysregulation of the IL-6 signalling pathway contribute to the development of inflammation-related diseases. It plays a central role in the link between chronic inflammation and cancer by promoting the maintenance of the tumor microenvironment, tumor growth, angiogenesis, metastasis and inhibiting tumor cell apoptosis, thereby ensuring tumor cell survival.

The IL-6R complex is composed of the IL-6Rα subunit and the gp130 subunit, which is involved in the assembly of several other cytokine receptors and is required for signal transduction. While gp130 is expressed in all cells, IL-6Rα expression is physiologically restricted to a few cell types, such as hepatocytes, epithelial cells and some leukocytes.

As a first step in IL-6-induced STAT3 activation, the cytokine binds to the IL-6Rα subunit and the resulting IL-6/IL-6Rα dimer binds to the gp130 subunit. As a consequence, the conformation of gp130 is altered and the IL-6/IL-6Rα/gp130 heterotrimer dimerizes through

the gp130 subunit. In the resulting hexamer complex, the intracellular portions of the two gp130 subunits are brought into close proximity, allowing cross-phosphorylation of constitutively linked Jak kinases.

The Jak kinases also phosphorylate tyrosine residues in the cytoplasmic portion of gp130. The resulting phosphotyrosine (pTyr) motifs serve as molecular docking sites for monomeric, non-phosphorylated STAT3 proteins via their SH2 domain. Once STAT3 is recruited, the transcription factor itself becomes a substrate for active Jak kinases: phosphorylation of the tyrosine residue at position 705 occurs. This is followed by homodimerization of STAT3. In some cases STAT1/STAT3 heterodimers may also be formed. Dimerization of STAT proteins takes place through a reciprocal interaction between the pTyr motifs and the SH2 domains of the two STAT molecules. Phosphorylated dimers are translocated through the nuclear pore complex by α and β importin proteins into the nucleus, where they bind to specific DNA response elements and directly regulate the expression of target genes.

Under normal conditions, STAT3 activation is a rapid and transient process. Tight regulation of the duration and strength of activation is essential for the prevention of pathological lesions and is facilitated by a complex network of negative regulators. Negative regulators of STAT3 activation include members of the PIAS, SOCS and PTP protein families. PIAS proteins inhibit STAT3 transcriptional activity by blocking DNA-binding activity of STAT3 and by promoting the recruitment of transcriptional co-repressors. SOCS proteins bind to the STAT3-binding domain of the IL-6/IL-6Rα/gp130 complex and thus inhibit the recruitment of STAT3 proteins. Finally, tyrosine phosphatases can dephosphorylate STAT3 proteins on the tyrosine side chain.

Serine phosphorylation of STAT3

In addition to the tyrosine residue, the STAT3 contains another phosphorylation site on the serine 727 residue. Several serine/threonine kinases, including Cdk1 and 5, as well as MAP kinases, are involved in the serine phosphorylation process. Data available in the literature suggest that serine phosphorylation may play both positive and negative regulatory roles in canonical STAT3 signalling, however, the mechanism of action is not well established and the data are often contradictory. Phosphorylation of the serine residue is required for maximal transcriptional activity, but it also promotes the dephosphorylation of pTyr-STAT3, thereby shortening the duration of STAT3 transcriptional activity. In addition, the phosphorylation of STAT3 serine also plays a critical role in the onset of M-phase of the cell cycle.

Role of STAT3 in oncogenesis

Physiological roles of activated STAT3 are extremely broad, regulating cell differentiation, proliferation, survival and death. Because STAT3 plays a central role in a wide range of physiological processes, its activation in normal cells is transient and tightly controlled. In contrast, persistent activation of STAT3 has been shown in a significant proportion of solid and haematological tumours, including leukaemia, breast, prostate and pancreatic cancers, as well as various melanomas.

Hanahan and Weinberg summarized the fundamental changes that occur during tumour transformation in eight points: (1) reduced growth factor requirement, cell proliferation is mediated by autocrine signalling; (2) insensitivity to proliferation inhibitory signals; (3) avoidance of apoptosis; (4) unlimited replication potential; (5) enhanced angiogenesis; (6) tissue invasion and metastasis; (7) altered metabolism; (8) avoidance of immune response. Sustained STAT3 activation promotes at least three of these hallmarks, proliferation, survival and angiogenesis, but it is now also shown to play a role in the EMT process and metastasis formation.

One possible reason for the dysregulation of the STAT3 signalling pathway is the elevated level of IL-6 detected in several tumor types, including uveal melanomas. Persistently (hyper)activated STAT3 promotes tumur progression via transcription of the proto-oncogenic c-myc and cell cycle regulatory cyclin D1 target genes. IL-6 also promotes tumor cell survival by enhancing the expression of anti-apoptotic proteins such as B-cell lymphoma 2 protein Bcl-2 homologous antagonist/killer (BAK), Bcl-2-associated protein X (Bcl-XL) and survivin through STAT3 activation.

Chelidonine

Alkaloids are naturally occurring, basic, mainly heterocyclic organic compounds found in plants that contain at least one nitrogen atom. Chelidonine belongs to the family of benzophenantridine alkaloids, whose members are composed of two aromatic and two alkane rings. It has previously been shown to inhibit cell growth and proliferation and induce necrosis and apoptosis in a variety of human tumor cell lines, including uveal melanoma cell lines. It has the ability to induce the intrinsic pathway of programmed cell death by enhancing the release of reactive oxygen radicals and reducing mitochondrial membrane potential.

Chelidonine inhibits tubulin polymerization and reduces the amount of microtubules in cells. Presumably, it is incorporated into the cholchicine-binding site, which disturbs the dynamic instability of the microtubule system, resulting in cell cycle arrest in the G_2/M phase.

The G_2/M block results in morphological changes such as the formation of the multipolar mitotic spindle and multinuclear giant cells, which are the main hallmarks of mitotic catastrophe. Because of its effects on microtubules, chelidonine is considered a potential microtubule-targeting agent.

OBJECTIVES

Based on previous results of our research group, chelidonine, a member of the benzophenantridine alkaloid family, induces apoptosis and necrosis in OCM-1 human uveal melanoma cells. Literature data suggest that chelidonine, similar to other microtubule-targeting agents, which are widely used in tumor therapy, disrupts dynamic instability of microtubules and arrests the cell cycle in the G_2/M phase. In addition, it has been shown that several MTAs interfere with tyrosine and serine phosphorylation of the STAT3 transcription factor.

Considering the above, our experiments were aimed at answering the following questions:

(1) what is the effect of chelidonine on the cell cycle of OCM-1 and OCM-3 uveal melanoma cells;

(2) whether chelidonine alters the expression of the members of IL-6/IL-6R/STAT3 signaling pathway and that of Bcl-2, an antiapoptotic protein regulated by STAT3;

(3) how chelidonine affects tyrosine and serine phosphorylation of STAT3 and whether there is any correlation between the effects on the two types of phosphorylation.

MATERIALS AND METHODS

OCM-1 and OCM-3 primary human uveal melanoma cell lines were used for our experiments. Both cell lines originate from malignant human uveal melanomas. The OCM-1 cell line has a spindle cell morphology, whereas the OCM-3 cell line has an epithelioid cell morphology. GNAQ and GNA11 mutations, which are common in human melanomas, are not present in either cell line, while BRAF (V600E) mutations were detected in both cell lines.

For alkaloid treatment, cells were cultured in the presence of 1 or 4 μg/ml chelidonine for the time periods appropriate to the experiment. Chelidonine was dissolved in DMSO. Before use the stock solution was serially diluted with the solvent so that the final concentration of DMSO was the same in all related samples. For control samples, the same amount of DMSO was added. After alkaloid treatment, the supernatant and cells were collected, washed and prepared for fluorescence labelling. The STAT3 transcription factor was activated with IL-6.

Cell viability, protein expression, phosphorylation and nuclear translocation of STAT3 were assessed by flow cytometry and/or confocal microscopy after labelling with fluorescent reagents.

Flow cytometry measurements were performed on a FACS Array or on a FACS Aria cytometer, while confocal microscopy measurements were performed on a Olympus Fluo View 1000 or a Zeiss LSM 880 microscope. Flow cytometry data were evaluated using the FCS Express program, while confocal microscopy images were analysed using FIJI and Matlab software. Spectrally different fluorophores were used for the simultaneous labelling of multiple cellular components. Possible aspecific binding of antibodies was monitored with irrelevant antibodies of the same isotype. Flow cytometric and/or confocal microscopy studies were performed within 24 hours of labelling.

The nuclear translocation of the STAT3 transcription factor was investigated using the "Trainable Weka Segmentation" module built into the FIJI image analysis software.

Student's t-test was used for statistical analyses using the Excel program. Changes were considered significant where $p<0.05$.

RESULTS

Chelidonine induces apoptosis and necrosis

In our experiments, we investigated the cell death-inducing effect of chelidonine by flow cytometric measurements based on DNA fragmentation and annexin/PI binding. Both methods confirmed that chelidonine induces cell death in human uveal melanoma cell lines in a concentration-dependent manner. Using annexin V/PI binding assays, we showed that chelidonine induced late apoptosis or necrosis in addition to apoptosis in a small proportion of cells.

Chelidonine arrests the cell cycle in the G_2/M phase and disrupts microtubules

Chelidonine resulted in significant G_2/M block in both cell lines, even with the shortest treatment. The proportion of cells arrested in the G_2/M phase increased with treatment duration. The alkaloid had a slower and more moderate effect on OCM 3 cells.

Using confocal microscopy, we revealed that chelidonine caused severe damage to the microtubule network, including the formation of abnormal mitotic spindle filaments and multiple spindle poles during mitosis. Furthermore, the appearance of multinucleated giant cells characteristic of mitotic catastrophe was also observed in the images.

Chelidonine inhibits IL-6-induced STAT3 activation

Next, we examined how chelidonine affects the efficiency of IL-6-induced activation, i.e. tyrosine phosphorylation, of STAT3 in the uveal melanoma cell lines. In control cells, activation of STAT3 could be detected in the vast majority of cells after IL-6 stimulation. A cell population with reduced IL-6 responsiveness appeared after 24 h of chelidonine treatment. The basal (constitutive) level of pTyr-STAT3, i.e. detectable in the absence of IL-6 stimulation, was not significantly affected by alkaloid treatment.

It is important to note that the effect of chelidonine followed an all-or-nothing principle: while pTyr STAT3 levels in low-responsive cells were reduced to basal levels, IL-6-induced activation of STAT3 in still-responsive cells remained at the levels observed in control cells.

Chelidonine decreases the expression of the gp130 subunit

In order to investigate what might underlie the chelidonine-induced reduced STAT3 activation, we examined the effect of chelidonine on the expression of certain members of the IL-6/IL-6R/STAT3 signalling pathway. After 24-hour chelidonine treatment, we observed a decrease in the expression of total STAT3, the IL-6α subunit and Jak2 in a negligible proportion of the cells. Therefore, it can be ruled out that the altered expression of these proteins is responsible for the reduced STAT3 activation.

During our measurements targeting the gp130 subunit, we found that the expression of gp130, although to different extents, was significantly reduced in the case of both cell lines as a result of chelidonine treatment. However, the expression of gp130 was only partially reduced, so it can be assumed that other factors are behind the inhibited tyrosine phosphorylation.

Chelidonine increases constitutive pSer-STAT3 levels

In our next experiments, we focused on another phosphorylation site of STAT3, the serine 727 residue. Our results show that, the basal serine phosphorylation of STAT3 can be detected even in the absence of IL-6 stimulation in both cell lines. In response to IL-6, the rate of serine phosphorylation was not or only slightly increased. In contrast to the constitutive pTyr-STAT3 level, which was not affected by the alkaloid, the level of constitutive serine phosphorylation of STAT3 was significantly increased in both cell lines upon chelidonine treatment. Similar to control cells, IL-6 stimulation did not or only negligibly alter the level of serine phosphorylation in chelidonine-treated cells.

Correlation study of reduced STAT3 activation and elevated pSer-STAT3 level

Chelidonine had opposite effects on STAT3 serine and tyrosine phosphorylation. Both effects affected only a fraction of cells, raising the question of whether there is an overlap between cell populations responding to alkaloid treatment by altering the levels of STAT3 tyrosine and serine phosphorylation. To address this question, the two phosphorylation sites of STAT3 were simultaneously labelled with monoclonal antibodies carrying spectrally distinct fluorophores, and samples were analysed by flow cytometry and confocal microscopy.

Our flow cytometry data show that after 24-hour chelidonine treatment, the vast majority of cells with increased basal pSer-STAT3 levels did not respond to IL-6 stimulation, i.e. there is an overlap between the involved cell populations. However, this overlap was only

partial, because the lack of response to IL-6 was also detected in cells that did not have elevated constitutive pSer-STAT3 levels in response to chelidonine, and in a minority of cells with elevated pSer-STAT3 levels, IL-6 was still able to induce STAT3 activation.

We also confirmed these findings by confocal microscopy and demonstrated by WEKA pixel-based segmentation that not only the level of pTyr-STAT3 but also its nuclear translocation was reduced in a significant proportion of cells with elevated pSer-STAT3 levels.

Chelidonine affects STAT3 phosphorylation in a time-dependent manner

We next examined the effect of chelidonine on STAT3 phosphorylation as a function of time. For this, in addition to the 24 h alkaloid treatment, two shorter time points (6 and 12 h) were used.

During the time-dependent measurements, we found that even the shortest (6 hours) chelidonine treatment increased the level of constitutive serine phosphorylation of STAT3 in a low but significantly higher proportion of cells compared to the control. We also showed an increased proportion of cells with reduced responsiveness to IL-6 stimulation after the shortest chelidonine treatment.

The proportion of cells not responding to cytokine stimulation increased monotonically with the duration of alkaloid treatment. In contrast, the fraction of cells with elevated pSer-STAT3 levels decreased after the initial increase (6 and 12 h), but remained significantly higher than in the control case.

It is important to note that after 6-hour treatment, a significant proportion of cells with elevated constitutive pSer-STAT3 levels showed IL-6-induced activation of STAT3, i.e. these cells retained their IL-6 responsiveness. However, the proportion of IL-6 stimulation-responsive cells with elevated level of pSer-STAT3 decreased monotonically with the increasing duration of alkaloid treatment.

Chelidonine increases Bcl-2 expression

We next examined whether the expression of the STAT3-regulated Bcl-2 protein is altered by chelidonine treatment of cells. We found that in the case of both cell lines chelidonine increased Bcl-2 expression in a significant proportion of cells, although to different extents. In OCM-1 cells, we observed a significant overlap between cell populations with increased Bcl-2 expression and decreased STAT3 activation. We also identified a small subset of cells in which, although Bcl-2 expression was unaltered, the IL-6 response was absent. Overlap of cell

populations with increased Bcl-2 expression and reduced STAT3 activation was also detected in OCM-3 cells. However, the extent of overlap is smaller, and the proportion of cells with unaltered Bcl-2 expression and reduced IL-6 responsiveness significantly exceeded the proportion of these cells.

DISCUSSION

In the experiments presented in this thesis, the effects of chelidonine, a member of the benzophenantridine alkaloids, on IL-6/IL-6R/STAT3 signalling were investigated in two human uveal melanoma cell lines by using methods that provide individual cell-by-cell information. Consistent with literature data, we show that chelidonine induces cell death and arrests the cell cycle in the G_2/M phase. Microscopic studies have demonstrated that chelidonine at low concentrations impairs the microtubule system and leads to the formation of abnormal mitotic spindles and multinucleated giant cells. These pathological lesions suggest the development of mitotic catastrophe, ultimately leading to apoptosis-like cell death.

The flow cytometric approaches combined with confocal microscopy that we used ensured a cell-by-cell comparison of two parameters and the discovery of overlaps between each affected population. Thus, we demonstrated that chelidonine exerts opposite effects on two types of STAT3 phosphorylation: it inhibits IL-6-induced tyrosine phosphorylation (STAT3 activation) but increases the level of STAT3 serine phosphorylation. The "all or nothing" principle applies to these effects, i.e. the phosphorylation status of STAT3 was either significantly changed or not affected at all.

The effects of chelidonine on two types of STAT3 phosphorylation followed different time kinetics over the examined time periods. The fraction of cells not responding to IL-6 stimulation (pTyr-STAT3^{LOW}) increased monotonically as a function of treatment duration. In contrast, the percentage of cells with increased constitutive pSer-STAT3 (pSer-STAT3^{HIGH}) levels followed a maximum curve: after an initial increase, it started to decrease again but remained significantly higher compared to the control samples after 24-hour treatment. Our results suggest that, while the effect on serine phosphorylation is transient, the inhibition of tyrosine phosphorylation is permanent, at least for the duration of our studies. The long-term effect of chelidonine on STAT3 activation is not due to reduced cell viability, as the percentage of cells not responding to IL-6 stimulation significantly exceeded the percentage of cells that died even at the higher alkaloid concentration (4 µg/ml).

The 24-hour chelidonine treatment inhibited IL-6-induced activation and nuclear translocation of the transcription factor in a significant proportion of pSer-STAT3^{HIGH} cells, which suggests a correlation of the effect on the two types of phosphorylation. However, it is important to note that after the shortest alkaloid treatment (6 hours), IL-6 stimulation was still able to induce STAT3 activation in the majority of pSer-STAT3 ^{HIGH} cells, and the lack of response to IL-6 was also detected in cells in which pSer -STAT3 level - at least apparently -

did not change under the influence of chelidonine. Our results suggest that chelidonine's interference with serine phosphorylation mechanisms prevents in time its effect in inhibiting tyrosine phosphorylation, and that elevated pSer-STAT3 levels alone are neither a sufficient nor a necessary condition for the absence of STAT3 activation. Thus, there is probably no direct causal link between increased pSer-STAT3 levels in response to chelidonine and inhibition of STAT3 activation, but further studies are needed to prove this.

Comparing our results with the literature, it appears that STAT3 serine phosphorylation involves at least two mechanisms that require the participation of different kinases and regulatory proteins. A certain constitutive level of pSer-STAT3 is required for STAT3 canonical functions, which ensures maximal STAT3 transcriptional activity and regulates the duration of STAT3 activation. In our own assays, phosphorylation of constitutive serine of STAT3 did not interfere with either STAT3 activation or its nuclear translocation.

The second mechanism may be responsible for the elevated pSer-STAT3 levels, which are part of the normal cell cycle during the initial stages of mitosis but can also be triggered or amplified by various stress effects. Chelidonine was presumably involved in the processes responsible for the second mechanism, without affecting the phosphorylation of the constitutive serine STAT3. It may have turned on and/or accelerated processes that positively regulate STAT3 serine phosphorylation (e.g. activation of Cdk1), but did not affect negative regulatory steps. The latter is supported by the transient nature of the effect.

The results of our time-dependent cell cycle measurements showed that the proportion of cells arrested in the G_2/M phase significantly exceeded the proportion of pSer-STAT3^{HIGH} cells during chelidonine treatment, regardless of the duration of treatment. These data confirm that increased serine phosphorylation of STAT3 is required for the initial phase of mitosis and is not a prerequisite for G_2/M block induced by chelidonine or chelidonine-like microtubule targeting agents.

We have shown that neither decreased cell viability nor increased pSer-STAT3 levels can be directly responsible for the effect of chelidonine on STAT3 activation. Our results also ruled out changes in IL-6Rα, Jak2 and total STAT3 expression as a cause of tyrosine phosphorylation inhibition. Although expression of the gp130 subunit is partially reduced by chelidonine, it is not completely abolished, suggesting that cells may still harbour functionally active IL-6 receptor complexes.

By our experiments targeting the expression of the anti-apoptotic protein Bcl-2, we found that chelidonine treatment resulted in a subpopulation of both cell lines with increased Bcl-2 expression. Our experiments to correlate Bcl-2 expression with pTyr-STAT3 levels

revealed that a significant proportion of the cell population with increased Bcl-2 expression had inhibited IL-6-induced activation of STAT3, similar to that observed for pSer-STAT3 levels. Further studies are needed to explore whether there is a relationship between elevated Bcl-2 and pSer-STAT3 levels.

The STAT3 transcription factor is a prominent target of anticancer therapies. Increasing evidence is emerging that inhibition of STAT3 activation sensitizes different types of tumour cells to radiotherapy and chemotherapy. Our results, together with literature data on microtubule-targeting agents, raise the potential applicability of chelidonine in combination/multimodal therapy of uveal melanomas and presumably other solid tumours. Our experiments also highlight that methods that provide cellular-level information can significantly advance our understanding of signalling processes. The combined use of flow cytometry and microscopy has allowed us to compare STAT3 serine and tyrosine phosphorylation on a cell-by-cell basis, to study their correlation, and to distinguish subpopulations that respond differently to IL-6 and chelidonine treatments, which is important for understanding the mechanism of antitumor action.

SUMMARY

In the work presented here, I investigated the effect of chelidonine on the IL 6/IL-6R/STAT3 signaling pathway in human uveal melanoma cell lines. Consistent with the literature, chelidonine induced cell death and trapped cells in the G_2/M phase of the cell cycle. Furthermore, chelidonine disrupted microtubules and led to the formation of abnormal microtubule filaments and multinucleated giant cells. These pathological changes suggest the development of mitotic catastrophe, which eventually leads to apoptosis-like cell death.

Chelidonine enhanced the efficiency of constitutive serine phosphorylation of STAT3 but abolished IL-6-induced activation (tyrosine phosphorylation) and nuclear translocation of the transcription factor. Biochemical techniques previously used to study STAT3 phosphorylation lacked the potential to provide cell-by-cell information. Flow cytometry combined with microscopy allowed us to compare serine and tyrosine phosphorylation of STAT3 on a cell-by-cell basis, to examine their correlation, and to distinguish subpopulations that respond differently to the alkaloid. In this way, we demonstrated that both effects are restricted to a fraction of cells only in an all-or-none fashion. While the loss of IL-6-inducible STAT3 activation was permanent over the time course of our experiments, the increase in pSer-STAT3 level appeared to be a transient effect. A partial overlap w observed between the affected subpopulations; however, no direct connection could be proven.

Chelidonine did not alter the expressions of IL-6Rα, Jak2 or STAT3. However, it reduced the level of gp130 in a significant proportion of cells. These cells, however, still expressed gp130, which, together with unchanged level of IL-6Rα, suggests that they still harbor functional IL-6R complexes.

We also observed the emergence of a subpopulation of cells with increased levels of Bcl-2, which overlaps with IL-6 unresponsive cells to a similar extent as observed in cells with increased levels of serine phosphorylated STAT3.

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List of publications related to the dissertation

1. **Csomós, I.**, Nagy, P., Filep, C. B., Rebenku, I., Nizsalóczki, E., Kovács, T., Vámosi, G., Mátyus, L., Dóczy-Bodnár, A.: Opposing Effects of Chelidonine on Tyrosine and Serine Phosphorylation of STAT3 in Human Uveal Melanoma Cells.
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11. Sárvári, A. K., Doan-Xuan, Q. M., Bacsó, Z., **Csomós, I.**, Balajthy, Z., Fésüs, L.: Interaction of differentiated human adipocytes with macrophages leads to trogocytosis and selective IL-6 secretion.
Cell Death Dis. 6, e1613, 2015.
DOI: http://dx.doi.org/10.1038/cddis.2014.579
IF: 5.378

Total IF of journals (all publications): 54,894
Total IF of journals (publications related to the dissertation): 9,627

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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