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Article

# Correlation of SERPINA-1 Gene Over-Expression with Inhibition of Cell Proliferation and Modulation of the Expression of IL-6, Furin, and NSD2 Genes

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**Abstract: Background and objectives:** IL-6 is a cytokine that promotes the proliferation of cancer cells by activating the JAK/STAT pathway via STAT3, which is expressed after methylation by NSD2. Furin, a pro-protein convertase responsible for the maturation of several precursor proteins such as pro-IGF-1R, involved in the activation of PI3K/AKT and the MAPK pathway, plays a crucial role in cancer proliferation. Our objective is to observe the impact of the overexpression of SERPINA-1, an anti-inflammatory and serine protease inhibitor, on cell proliferation and the genetic expression of IL-6, Furin, and NSD2. **Materials and Methods:** In hetero-hybrid lymphoblastoid cells either transfected with the SERPINA-1 gene (JP7pSer+) or without the SERPINA-1 gene (JP7pSer-); we studied by qRT-PCR the genetic overexpression of the SERPINA-1 gene and the genetic expression of Furin, IL-6, and NSD2 genes. Cell proliferation was tested by colorimetric and enzymatic methods, and the membrane expression of IGF-1R was measured by flow cytometry. **Results:** The proliferation of the JP7pSer+ cell line is decreased by 91% and 85% in both measurement methods on day 6 compared to JP7pSer- cells. The kinetics of the genetic expression of Furin, IL-6, and NSD2 show an increase in expression times in JP7pSer+ cells compared to JP7pSer- cells, but after three days, this ratio is reversed. The ratio of mean fluorescence intensity shows that JP7pSer- cells express 1.33 times more IGF-1R than JP7pSer+ cells. **Conclusion:** These results suggest promising therapeutic avenues for cancer, which should be complemented by advanced studies on the expression of SERPINA-1 and the interaction with the expression of IL-6 and Furin.

**Keywords:** SERPINA-1; hetero-hybrid lymphoblastoid; Furin; IL-6; NSD2; IGF-1R

## 1. Introduction

Cancer is a complex disease characterized by uncontrolled cell proliferation, driven by a combination of genetic, environmental, and epigenetic factors [1]. The cellular signalling pathways are central to these events, which regulate cell growth, survival, and differentiation [2,3]. These pathways are complex networks of proteins involved in genetic and epigenetic regulation, as well as in modulating the cellular environment, influencing fundamental processes such as apoptosis, cell proliferation, and tissue invasion [3–5]. Prominent signalling pathways frequently deregulated in cancer development include the PI3K/AKT/mTOR, MAPK/ERK, and JAK/STAT pathways [6–9].

These pathways are often abnormally activated by genetic mutations or aberrant activation mechanisms, leading to excessive stimulation of proliferation and inhibition of apoptosis mechanisms [10–12]. Indeed, the JAK/STAT pathway, when activated by IL-6, a cytokine implicated in several cancer [9], induces the activation of the transcription factor Stat3 in tumor cells. The activation of the NF- $\kappa$ B and Stat3 signalling pathways promotes resistance to apoptosis,

angiogenesis, and metastasis of tumor cells [13,14]. Furthermore, IL-6 contributes to the resistance of tumor cells to treatment, such as chemotherapy, by promoting survival and invasion and by inhibiting apoptosis of malignant cells [9,15].

IL-6 plays a role in modulating the tumor microenvironment, promoting inflammation and tumor growth, such as in multiple myeloma [16,17]. Song and colleagues demonstrated that NSD2 can methylate and activate STAT3, creating a potential positive feedback loop that amplifies IL-6 signalling [18]. NSD2 is a methyltransferase that plays a key role in the regulation of gene expression by methylating histones. This enzyme is responsible for the catalysis of mono- and di-methylation of lysine 36 on histone H3 [19]. This histone modification can recruit or exclude other chromatin-associated proteins, leading to either activation or repression of gene expression, depending on the cellular context [20]. NSD2 expression is frequently elevated in various types of cancers [18,21]. The evolution of cancer involves complex biological and environmental events that depend on the activity of various enzymes. Among these, proprotein convertases, particularly Furin, are involved in the maturation of numerous precursor proteins into their active forms, thereby promoting the progression of several cancers [22,23]. Furin, a subtilisin-like protease, cleaves precursor proteins at the consensus site of Arg - X - Lys/Arg - Arg or Arg - X - X - Arg, facilitating their maturation [24–26].

Furin is involved in the maturation of precursors such as pro-TNF- $\alpha$ , pro-TGF- $\beta$ 1, and pro-IGF-1R, as well as various growth factors. These molecules play important roles in diverse cellular processes, including inflammation, growth, and angiogenesis, which are frequently deregulated in cancer [26–28]. In the context of the tumor microenvironment, pro-inflammatory cytokines matured by Furin, such as pro-TNF- $\alpha$  and pro-TGF- $\beta$ 1, can induce the activation of IL-6 [29], which in turn promotes tumor growth, angiogenesis, and metastasis [13]. Indeed, IL-6 produced by tumor microenvironment cells induces the activation of the JAK/STAT3 pathway in tumor cells, stimulating their proliferation, survival, and invasion [9]. Furin, therefore, contributes to the regulation of several signalling pathways crucial for tumor progression, including the maturation of the pro-IGF-1R precursor into IGF-1R, enabling its expression on the cell surface [30,31]. By interacting with its ligands IGF1 and IGF2, IGF-1R activates cell proliferation and, in certain cancers, promotes tumor progression [32]. Furin is often overexpressed in various cancers, suggesting its potential role in the survival and proliferation of tumor cells [26].

Understanding the molecular mechanisms of signalling pathways involving inflammatory processes mediated by cytokines just like IL-6 and maturation by Furin, opens new avenues for the development of targeted therapies aimed at restoring the normal regulation of cellular processes. In doing so, it is essential to explore not only individual signalling pathways but also their interactions and their roles within the tumor microenvironment. Thus, the study of signalling pathways in the context of cancer is essential for the development of innovative diagnostic and therapeutic strategies. However, the involvement of Furin in different cancers, such as malignant haematological diseases, remains largely unknown, particularly regarding its potential interactions with IL-6 and NSD2. Studies on the links between these markers would help identify common mechanistic pathways and specificities in different cancer pathologies. To date, no study has established a definitive mechanistic link between these three players (Furin, IL-6 and NSD2) in cancer, which limits the understanding of their synergies or common regulatory pathways. One in vitro approach is to verify whether there are significant interdependencies or correlations between the expression of Furin, IL-6, and NSD2 by inhibiting one of the three elements. Alpha-1 antitrypsin (A1AT), encoded by the SERPINA1 gene, is an inhibitor of serine proteases, particularly neutrophil elastase, which is its main target [33]. Likewise, Alpha-1 PDX is a variant of A1AT, a serine protease inhibitor, targeting other proteases such as Furin, PACE4, and PC7 [34]. Our objective is to assess the effect of the SERPINA-1 overexpression on these Furin, IL-6, and NSD2 markers in a proliferative hetero-hybrid lymphoblastoid cell model.

## 2. Materials and Methods

### 2.1. Maintenance of the JP7 Cell Line

The JP7 cell line, established in our laboratory according to the protocol described by Tissot and al. [35], originated from the transformation of human lymphocytes by the Epstein-Barr virus. These transformed lymphocytes were then stabilized by fusion with the murine X63Ag8 myeloma cell line derived from BALB/c mice. The resulting hetero-lymphoblastoid cells are maintained in continuous culture through successive passages in RPMI 1640 culture medium supplemented with 10% foetal bovine serum (FBS), 200 mM glutamine, and 100 IU/ml penicillin and streptomycin. Cultures are maintained in sterile containers and incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>.

## 2.2. Establishment, Identification, and Selection of JP7pSer<sup>+</sup> and JP7pSer<sup>-</sup> Cell Lines

JP7 cells were cultured in a 6-well plate at a density of  $5 \times 10^5$  cells/well in 3 ml of culture medium. After 24 hours, the cells were washed and supplemented with 1 ml/well of culture medium containing 1 µg of either the pCMV6-SERPINA1-GFP or pCMV6-GFP plasmid vector, along with 8 µl of transfection reagent (CANFAST, Canvaxbiotech). Cells transfected with pCMV6-SERPINA1-GFP were designated JP7pSer<sup>+</sup>, while cells transfected with pCMV6-GFP were designated JP7pSer<sup>-</sup>. Following a 5-hour incubation period, the cells were washed by centrifugation with RPMI medium without FBS and returned to culture as described above, with the addition of the selective antibiotic G418 at 400 µg/ml. After 48 hours, the cells were harvested for downstream assays. Cell condition and proliferation were monitored by microscopy (EVOS™ FL Digital Inverted Fluorescence Microscope). Under these selective conditions, only transfected cells that had integrated the G418 resistance gene and expressed GFP were able to proliferate, as indicated by green fluorescence. Transfection efficiency was assessed by determining the percentage of fluorescent cells using microscopy (EVOS™ FL Digital Inverted Fluorescence Microscope) at 200-x magnification, and SERPINA1 gene expression was confirmed by qRT-PCR.

## 2.3. Evaluation of the Effect of Serpina-1 Gene Transfection on Cell Lines in Culture

### 2.3.1. Cell Proliferation

The colorimetric study using Trypan blue exclusion,  $20 \times 10^3$  cells/well were seeded into six 96-well plates. For the enzymatic study employing 3-(4,5-diméthyl-2-thiazolyl)-2,5-diphényl-2H-tétrazolium (MTT),  $5 \times 10^3$  cells/well were seeded into three 96-well plates. The JP7pSer<sup>+</sup> and JP7pSer<sup>-</sup> cells were cultured for 6 days under standard conditions as previously described, but without the selective G418. The culture medium was not renewed during this period. Cell viability and proliferation were assessed daily and in parallel using both the Trypan blue exclusion and MTT assays. All tests were performed in triplicate across three independent experiments. The percentage of viable cells, as determined by Trypan blue exclusion, was quantified by microscopy using a Malassez counting chamber. The enzymatic activity of the cells was evaluated by spectrophotometric measurement at 570 nm, which quantifies the optical density of the formazan product resulting from the reduction of MTT by succinate dehydrogenase, a mitochondrial metabolic enzyme.

### 2.3.2. Gene Expression of Serpina-1, Furin, IL-6 and NSD2

The total RNA from the cell lines was extracted using the kit (PRimeZOL RNA Isolation, Canvaxbiotech), in accordance with the manufacturer's instructions, and quantified by spectrophotometry (MaestroNano). A quantity of 250 ng/µl of RNA was used for each qRT-PCR using the kit one-step (qMAXSen, Canvaxbiotech). The specific primers used for the genes, SERPINA-1 (Forward: 5'-GGCTGACACTCACGATGAAA-3'; Reverse: 5'-GTGTCCCCGAAGTTGACAGT-3'), FURIN (Forward: 5'-GCCCAGAATTGGACCACAGT-3'; Reverse: 5'-TCCCGATGTCTTTGGGCTC-3'), IL-6 (Forward: 5'-AGACAGCCACTCACCTCTTCAG-3'; Reverse: 5'-TTCTGCCAGTGCCTCTTTGCTG-3'), NSD2 (Forward: 5'-AATATGACTCCTTGCTGGAGCAGG-3'; Reverse: 5'-ATTTCAACAGGTGGTCTTTGTCTC-3') and Beta-actin (Forward: 5'-TGGAACTCTGTGGCATCCATGAAAC-3'; Reverse: 5'-TAAAACGCAGCTCAGTAACAGTCC-3') provided and reconstituted according to the manufacturer's recommendations (NeoBiotech).

Amplification in 35 cycles was performed in a thermocycler (BIO-RAD CFX 96, C1000) calibrated for SYBR Green fluorescence quantification. The qRT-PCR experiments were performed in triplicate daily for 5 days. The results obtained were normalized separately to the human beta-actin reference gene according to the Livak method before calculating the means of the observed expression ratios each day [36].

### 2.3.3. Membrane Expression of the IGF-1 Receptor

The membrane expression of IGF-1R was measured by flow cytometer (BD FACS Lyric Flow Cytometry System, BD Biosciences). For this purpose, the JP7pSer<sup>+</sup> and JP7pSer<sup>-</sup> cells, suspended at  $30 \times 10^3 / 100 \mu\text{l}$  in RPMI-1640 medium without additives, were incubated in 5 ml tubes with  $10 \mu\text{l}$  of the anti-CD221-PE monoclonal antibody (Clone IH7, Origène Technologie) or the non-specific anti-IgG1-PE isotype antibody (Clone HP6001, BD Pharmingen). After 30 minutes of incubation in the dark at room temperature, the cells were washed 3 times by centrifugation with RPMI-1640 medium and then suspended for flow cytometer analysis.

### 2.4. Statistical Analysis

The normality of the distribution of values was verified by the Shapiro-Wilcoxon test. Statistical differences according to the t test, Welch and Fisher tests were verified using Graph Pad Prism8 software.

## 3. Results

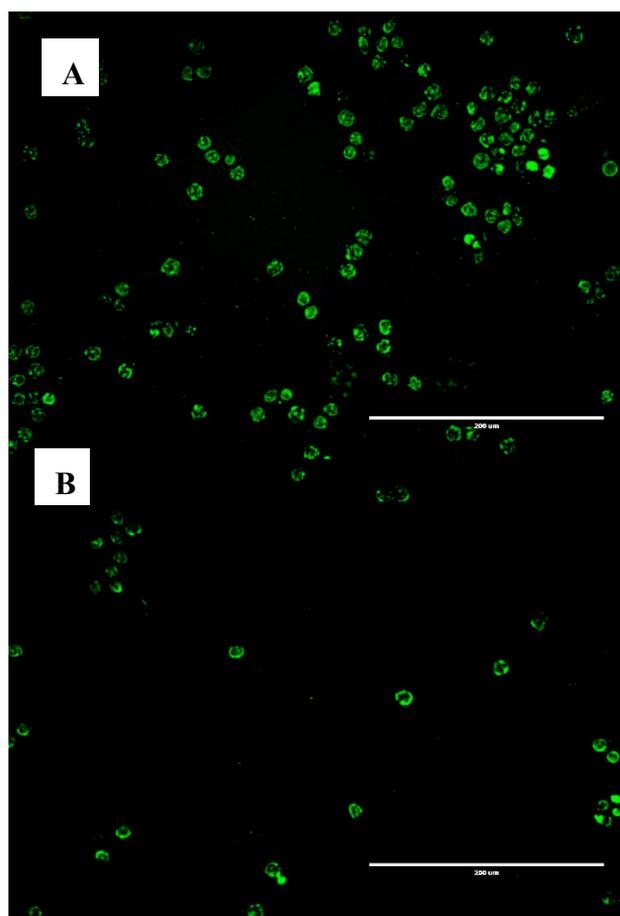
### 3.1. Establishment, Identification, and Selection of JP7pSer<sup>+</sup> and JP7pSer<sup>-</sup> Cell Lines

The selection of transfected cell lines was based on observations from both fluorescence microscopy and the quantification of mRNA for A1AT. Culture wells containing fluorescent cells at greater than 95% and an SERPINA1 gene mRNA level at least equal to 1 relative to the beta-actin reference gene were selected.

#### 3.1.1. Microscopy

To evaluate the stability of the JP7pSer<sup>-</sup> and JP7pSer<sup>+</sup> model lines, the cells were observed under a fluorescence microscope and digitized to have more than 95% of the fluorescent cells, in order to perform the genetic expression and proliferation tests.

After 48 hours of culture with selective agent G418 to generate stable cell lines, more than 95% of JP7pSer<sup>+</sup> and JP7pSer<sup>-</sup> cells emitted green fluorescence under excitation at 490 nm (blue light). These results show that JP7pSer<sup>+</sup> are fluorescent and express GFP, this expression is indicative of A1AT expression in the JP7pSer<sup>+</sup> line because the same cytomegalovirus promoter governs both the SERPINA-1 and GFP genes (Figure 1).

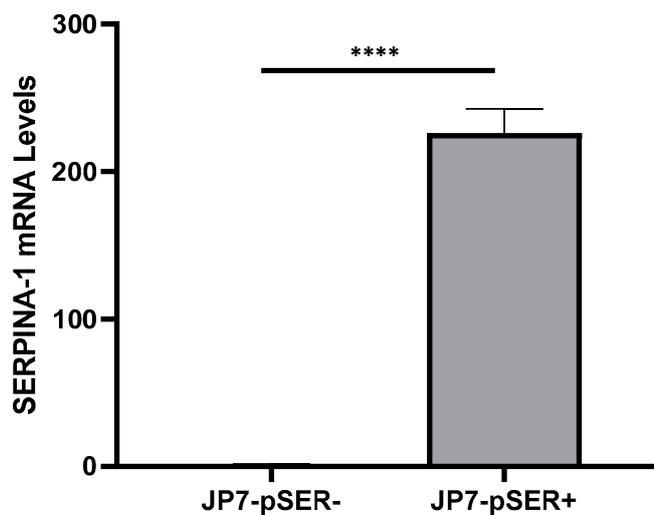


**Figure 1.** JP7pSer<sup>-</sup> (A) and JP7pSer<sup>+</sup> (B) cells observed under a microscope at 200x magnification. The majority of the cells under 490 nm excitation emits the green fluorescence of the GFP protein. Scale 200 $\mu$ m.

### 3.1.2. qRT-PCR

qRT-PCR analysis was performed on several wells of cells to measure SERPINA-1 gene expression in both JP7pSer<sup>+</sup> and JP7pSer<sup>-</sup> cell clones in order to confirm SERPINA-1 overexpression by JP7pSer<sup>+</sup> cells. Beta-actin was used as the housekeeping gene in this experiment.

The average expression of SERPINA-1 gene in JP7pSer<sup>-</sup> cells is 1.2, while that of JP7pSer<sup>+</sup> cells is 226, relative to the expression of the Beta-actin housekeeping gene (Figure 2). These results therefore confirm the overexpression of the SERPINA-1 in JP7pSer<sup>+</sup> cells line.



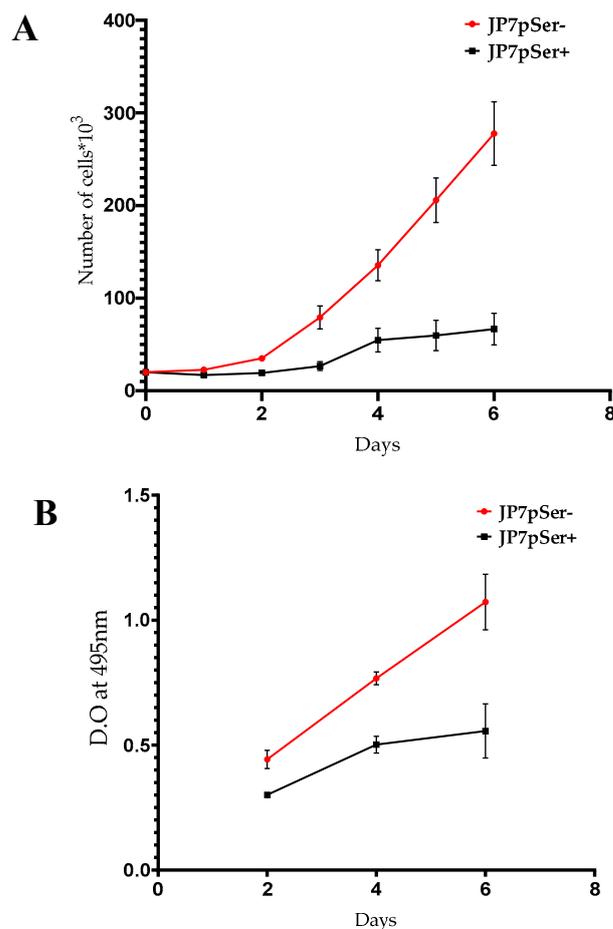
**Figure 2.** Relative expression of the SERPINA-1 gene in JP7pSer- and JP7pSer+ cells referred to the expression of Beta-actin housekeeping gene. Data are mean  $\pm$  SD of at least three independent experiments and significant differences between different groups were shown as \*\*\*\* p-value < 0.0001.

### 3.2. Evaluation of the Effect of SERPINA1 Gene Transfection on Cultured Cell Lines

#### 3.2.1. Cell Proliferation

The proliferation test was designed to determine whether the proliferative capacity of JP7pSer+ cells overexpressing SERPINA-1 is lower than that of JP7pSer- cells.

To confirm this, two proliferation tests were used: a colorimetric method based on counting viable cells with trypan blue (Figure 3-A), and an enzymatic method by MTT based on the metabolic activity of the cells (Figure 3-B).



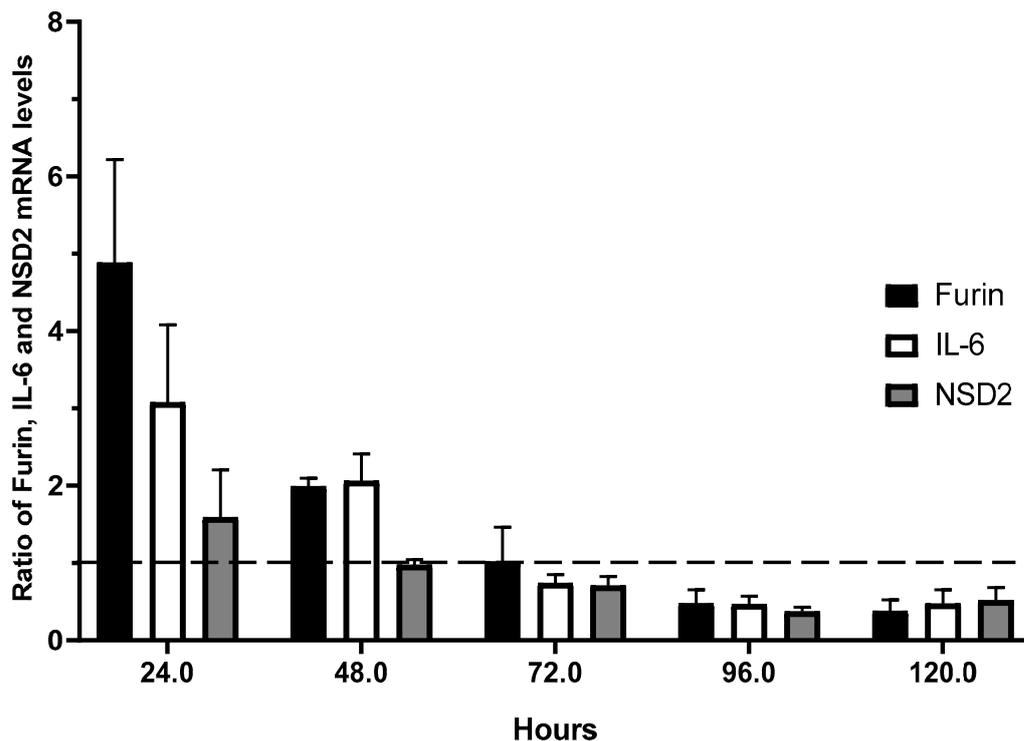
**Figure 3. (A)** The proliferation of  $20 \times 10^3$  cells/well of JP7pSer+ or JP7pSer- evaluated by the colorimetric Trypan blue method. **(B)** The proliferation of  $5 \times 10^3$  cells/well of the two cell lines, measured by enzymatic method (MTT). O.D: Optical density. Data are mean  $\pm$  SD of at least three independent experiments and significant differences between different groups with  $p < 0.05$  for both experiments.

The colorimetric proliferation assay of JP7pSer+ cells shows the same trends, with a decrease in proliferation of more than 64% in 4 days and more than 91% after 6 days (Figure 3-A). The proliferation measured by MTT of JP7pSer+ cells decreases by 38% in 4 days and by more than 85% in terms of 6 days compared to JP7pSer- cells (Figure 3-B).

### 3.2.2. Kinetics of Gene Expression for Furin, IL-6, and NSD2

The kinetic study of the expression by qRT-PCR of Furin, IL-6 and NSD2 was designed to observe the inhibitor effect of the SERPINA1 gene on JP7pSer+ cells compared to JP7pSer- cells. These gene expressions of Furin, IL-6 and NSD2 were calculated using the Livak and Schmittgen method [36], relative to the expression of the Beta actin gene.

The qRT-PCR analysis revealed that the JP7pSer+/JP7pSer- ratio of Furin gene expression was 4.88 on the 1st day and became 1.01 on the 3rd day, decreasing to 0.37 on the 6th day of culture. However, the same cellular ratio of IL-6 gene expression was 3.07 on the 1st day and decreased to 0.74 on the 3rd day, reaching 0.47 on the 6th day. As for NSD2 expression, the JP7pSer+/JP7pSer- ratio was 1.59 on the 1st day and decreased to 0.7 on the 3rd day, continuing to decrease to 0.51 on the 6th day (Figure 4). Three experiments were conducted to study the effect of the SERPINA1 gene over-expression on the expression kinetics of the three genes encoding Furin, IL-6, and NSD2. All three experiments yielded reproducible and highly similar results.



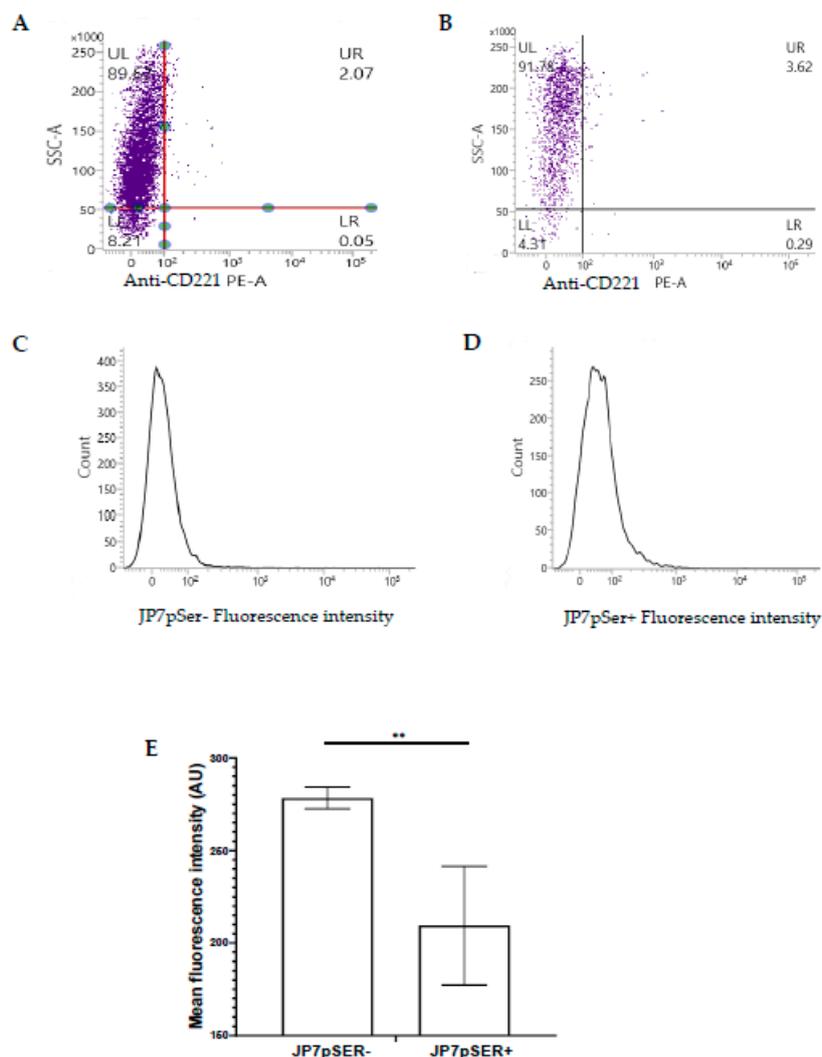
**Figure 4.** Kinetics of the Furin, IL-6 and NSD2 gene expression measured by qRT-PCR in the JP7pSer+ line referred to that of the control line JP7pSer-. Data are mean  $\pm$  SD of at least three independent experiments. Furin :  $Y = -0,04386X + 4,908$  ;  $R^2 = 0,7251$  ;  $r = -0,85 \in CI95$  ;  $p=0,0001$ . IL-6 :  $Y = -0,02834X + 3,404$  ;  $R^2 = 0,7463$  ;  $r = -0,86 \in CI95$  ;  $p=0,0001$ . NSD2 :  $Y = -0,01150X + 1,661$  ;  $R^2 = 0,6295$  ;  $r = -0,79 \in CI95$  ;  $p=0,0004$ .

These results indicate an influence of SERPINA-1 gene over-expression on the kinetics of Furin, IL-6, and NSD2 expression, with an initial increase in expression followed by a progressive decrease.

### 3.2.3. Membrane Expression of the IGF-1 Receptor (IGF-1R, CD221)

This section will likely examine the inhibitor effect of SERPINA1 gene on the surface expression of the Insulin-like Growth Factor 1 Receptor (IGF-1R), also known as CD221.

Cytofluorimetric analysis using anti-CD221-PE showed a near-absence of labelling in JP7 cells. The JP7pSer- and JP7pSer+ cell lines showed low and nearly similar percentages with JP7pSer- (3.9%) vs JP7pSer+ (3.46%),  $t$ -test = 1.2 with a  $p$ -value = 0.22 > 0.05, not significant. The average fluorescence intensity over three measurements for each cell line shows that JP7pSer- cells have an average MFI=278.33 AU, while for JP7pSer+ the average MFI=209.33 AU, with different confidence intervals CI99: [272.48; 284.17] for JP7pSer- and CI99: [177.31; 241.34] for JP7pSer+. The  $p$ -value < 0.01 with a significance threshold of 0.05. These results indicate that CD221 expression is globally very low in both JP7pSer- and JP7pSer+ cell lines, but with a 25% decrease in JP7pSer+ compared to JP7pSer- (Figure 5-E).



**Figure 5.** Graphs of one of the three flow cytometry measurements analysis of membrane expression of IGF-1R by JP7pSer- (A) Vs JP7pSer+ (B) cells, and fluorescence intensity of JP7pSer- (C) Vs JP7pSer+ (D). Mean fluorescence intensity (MFI) was calculated from the three measurements (E), and the average MFI was 278.33 AU for JP7pSer- cells with a CI99: [272.48; 284.17], and an average MFI of 209.33 for JP7pSer+ cells with a CI99: [177.31; 241.34]. The \*\*p-value < 0.01, with a significance threshold of 0.05.

#### 4. Discussion

The JP7 cell line, derived from a fusion between an EBV-transformed lymphocyte and a murine myeloma, was used to study the effects of SERPINA-1 gene expression and its product, alpha-1-antitrypsin (A1AT), on cell proliferation and the expression of Furin, IL-6, and NSD2 genes. JP7pSer+ cells, overexpressing SERPINA-1, but not JP7pSer- cells, were generated using the same plasmid under the control of the cytomegalovirus promoter. This approach allowed us to isolate the effect of SERPINA-1 overexpression while maintaining identical experimental conditions for both cell lines. This model helps to study the effect of A1AT, on different cellular processes. A1AT, a potent anti-inflammatory protein and a major serine proteinase inhibitor, is mainly synthesized by hepatocytes and highly expressed during inflammatory [37]. In our study, SERPINA-1 mRNA expression in JP7pSer+ cells, measured 48 hours after transfection several times, showed levels ranging from 208 to 240 times higher than those in the JP7pSer- cell line. Previous studies have reported that gene expression under the control of the CMV promoter can vary from 10 to 1000-fold depending on the cell type and the number of integrated promoters, as well as the number of plasmids transfected per

cell [38–40]. However, in the JP7pSer<sup>-</sup> and JP7pSer<sup>+</sup> cell lines, IGF-1R expression is present in nearly 3.9% on the cell surface, suggesting that this signalling pathway may not play a major role in cell proliferation [41], probably due to the activation of the CMV promoter, which increases Furin expression and allows for the cleavage of pro-IGF-1R. In contrast, in the JP7pSer<sup>+</sup> cell line, the mean fluorescence intensity of IGF-1R expression was reduced by 25%, suggesting that SERPINA-1 overexpression inhibits Furin, thereby preventing the cleavage of pro-IGF-1R and reducing the availability of IGF-1R on the cell surface [42–44]. Thus, this reduction in IGF-1R expression on the surface of JP7pSer<sup>+</sup> cells provides an indirect argument in favor of the biological activity of A1AT, demonstrating not only its impact on a specific signaling pathway but also on cell proliferation as assessed by enzymatic and colorimetric [45,46]. Indeed, after 72 hours, the proliferation of JP7pSer<sup>+</sup> cells dropped by more than 50%, and then by more than 90% after 96 hours, while that of JP7pSer<sup>-</sup> cells remained stable at 90%. To understand the molecular effects during this progressive decrease in proliferation, we analyzed the mRNA expressions of Furin, IL-6, and NSD2. This study revealed a significant negative correlation between the gene expression ratios over time for the three genes of interest, Furin, IL-6, and NSD2. Initially high, these ratios decreased to levels close to one after 72 hours of culture, likely reflecting an initial adaptive response followed by a negative regulation of the Furin, IL-6, and NSD2 genes in the JP7pSer<sup>+</sup> line. The indirect relationship between IL-6 and Furin observed in JP7pSer<sup>+</sup> cells can be attributed to the role of IL-6 as an inflammatory mediator, which can induce Furin expression under certain conditions. This is consistent with studies demonstrating that IL-6 plays a dual role, both in pro-inflammatory and pro-tumorigenic processes, depending on the cellular context and the signaling pathways [47]. This regulation may also be involved in controlling the growth and survival of tumor cells, as shown by research on the role of IL-6 in the tumor [13].

In contrast, the JP7pSer<sup>-</sup> cell line maintained a more stable gene expression, highlighting the specific role of A1AT in the regulation of these genes. Furthermore, linear regression data showed strong and significant correlations between Furin and NSD2, as well as between Furin and IL-6, suggesting that Furin is a central point in the regulation of these genes. However, these correlations do not establish a direct causal relationship. The correlation between NSD2 and IL-6 indicates a potential interconnection between these pathways, involving epigenetic and inflammatory mechanisms.

## 5. Conclusion

The overexpression of the SETRPINA-1 gene inhibits cell proliferation and modulates the expression of NSD2, IL-6, and Furin. The reduction in CD221 expression in JP7pSer<sup>+</sup> cells reinforces the hypothesis that Furin inhibition plays a central role in these effects. These results open avenues of research for potential therapeutic targets. However, further molecular studies are needed to confirm these hypotheses and explore the functional implications of these interactions.

**Author Contributions:** Conceptualization, NASSIM TASSOU, AHMED TISSENT and NORDDINE HABTI; Methodology, NASSIM TASSOU and NORDDINE HABTI; Validation, AHMED TISSENT and NORDDINE HABTI; Formal analysis, NASSIM TASSOU and NORDDINE HABTI; Resources, NASSIM TASSOU and HAJAR ANIBAT; Data curation, NASSIM TASSOU; Writing – original draft, NASSIM TASSOU; Writing – review & editing, NASSIM TASSOU and NORDDINE HABTI; Visualization, NASSIM TASSOU and AHMED TISSENT; Supervision, NORDDINE HABTI; Project administration, NORDDINE HABTI; Funding acquisition, HAJAR ANIBAT and NORDDINE HABTI.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

IGF-1	Insulin-like growth factor-1
PI3K	Phosphatidylinositol 3-kinase
AKT	Protein kinase B
mTOR	Mammalian target of rapamycin
MAPK	Mitogen-activated protein kinase
ERK	Extracellular signal-regulated kinases
JAK	Janus kinases
STAT	Signal transducer and activator of transcription proteins
IGF-1R	Insulin-like growth factor-1 receptor
IL-6	Interleukin 6
TNF- $\alpha$	Tumor necrosis factor
NSD2	Probable histone-lysine N-methyltransferase
EBV	Epstein-Barr virus
STAT3	Signal transducer and activator of transcription 3
Lys	Lysine
Arg	Arginine
TGF- $\beta$ 1	Transforming growth factor beta 1
PC	Proprotein convertase
A1AT	Alpha-1 antitrypsin
Alpha-1PDx	alpha-1 antitrypsin Portland variant
PACE4	Proprotein convertase subtilisin/kexin type 6
PC7	Proprotein convertase subtilisin/kexin type 7
CMV	Cytomegalovirus
GFP	Green fluorescent protein
mRNA	Messenger ribonucleic acid
qRT-PCR	Quantitative Reverse Transcription – Poly Chain Réaction
CI	confidence interval
mRNA	messenger ribonucleic acid

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