European Journal of Biology
Eur J Biol 2024; 83 (2): 213–221
DOI: 10.26650/EurJBiol.2024.1531120

RESEARCH ARTICLE

Notch and Hedgehog Signalling Axis Drive Senescence in HER2-Positive Breast Cancer Resistant to Trastuzumab

Asiye Busra Boz Er[1], Idris Er[2]

[1]Recep Tayyip Erdogan University Faculty of Medicine, Department of Medical Biology, Rize, Türkiye
[2]Karadeniz Technical University Faculty of Medicine, Department of Medical Biology, Trabzon, Türkiye

ABSTRACT

Objective: Cellular senescence halts the proliferation of damaged or preneoplastic cells, playing a vital role in cancer control. In HER2-positive breast cancer, resistance to trastuzumab, a HER2-targeted monoclonal antibody, remains a significant obstacle. Although the trastuzumab and cilengitide combination reduces stemness and epithelial-mesenchymal transition, its effect on senescence remains unclear. Additionally, inhibiting the Notch and Hedgehog pathways can induce senescence by impairing proliferation, stemness, and cell cycle progression, making them promising therapeutic targets. This study aimed to evaluate the effect of trastuzumab/cilengitide on cellular senescence in HER2-positive trastuzumab-resistant breast cancer cells and to elucidate the roles of Notch and Hedgehog signalling in this process.

Materials and Methods:: HER2-positive breast cancer cell lines HCC1954 and SKBR3, along with their trastuzumab-resistant variants, were treated with trastuzumab, cilengitide, or both. Senescence markers were assessed by real-time PCR. Notch and Hedgehog pathway activity was evaluated, with additional experiments using specific inhibitors Fli06 (Notch) and GANT61 (Hedgehog).

Results: The trastuzumab-cilengitide combination significantly upregulated senescence markers relative to monotherapy. This response was associated with a marked decrease in Notch and Hedgehog pathway activity. Further combined inhibition of these pathways enhanced senescence marker expression, underscoring their involvement in drug-induced senescence.

Conclusion: The trastuzumab-cilengitide combination induces senescence in trastuzumab-resistant HER2-positive breast cancer cells, potentially through Notch and Hedgehog inhibition. These findings support targeting senescence pathways as a novel strategy to overcome trastuzumab resistance and improve therapeutic outcomes. Further research is warranted to assess the clinical potential of such combination therapies.

Keywords: HER2-positive, Senescence, Notch, Hedgehog.

INTRODUCTION

Throughout life, cells encounter various types of damage. Depending on the severity and nature of this damage, cells can either repair themselves or activate death signalling pathways to prevent disruption of tissue homeostasis.[1] When repair mechanisms are insufficient, cells activate apoptotic signalling pathways to eliminate damaged cells and preserve tissue homeostasis. Alternatively, cells may undergo cellular senescence, a robust and irreversible cell cycle arrest, triggered by diverse stress signals. This response acts as a safeguard against the replication of aged, damaged, or preneoplastic cells, thereby limiting their potential to contribute to tumorigenesis.[2] In our previous work, we demonstrated that the combination of trastuzumab and cilengitide significantly decreased stemness[3] and epithelial-mesenchymal transition (EMT)[4] in both HER2-positive trastuzumab-resistant and -sensitive cell lines. However, the effects of this combination on senescence are unknown. Cilengitide is an arginine-glycine-aspartic acid (RGD)-containing pentapeptide that effectively blocks αvβ3 and αvβ5 integrin activation.[5] The impact of RGD-binding integrins on cellular senescence is still debated due to their presence in different combinations.[6] Recent studies have shown that CWHM12, an RGD-mimicking integrin blocker, increases senescence in hepatic stellate cells by binding to and inhibiting RGD-binding integrins.[7-12] However, the effect of RGD-binding integrin inhibitors on HER2-positive breast cancer remains unexplored. In this study, the effect of cilengitide on the

Corresponding Author: Asiye Busra Boz Er **E-mail:** asiyebusra.bozer@erdogan.edu.tr
Submitted: 09.08.2024 • **Revision Requested:** 19.09.2024 • **Last Revision Received:** 23.10.2024 • **Accepted:** 11.11.2024

senescence of HER2-positive trastuzumab-resistant cell lines is to be investigated, and the underlying signalling mechanisms are to be elucidated.

Notch and Hedgehog signalling pathways, which are essential for development and tissue homeostasis, also significantly affect senescence in cancer cells, affecting tumour progression and therapeutic outcomes. The activation of Notch signalling pathways can induce senescence in various cancers. Notch receptors such as Notch1 and Notch2 promote senescence through the upregulation of cyclin-dependent kinase inhibitors (CDKIs) such as $p16^{INK4a}$ and $p21^{CIP1}$.[4,5] For instance, Notch activation in melanoma and breast cancer models increases $p21^{CIP1}$ expression, leading to growth arrest and senescence induction, thereby inhibiting tumour progression.[13] Conversely, in some contexts, Notch signalling may suppress senescence and promote cancer progression. In glioblastoma and certain leukaemia cells, Notch activation correlates with enhanced cell survival and proliferation, suggesting a role in evading senescence-associated growth arrest.[14]

In previous studies Hedgehog signalling activation associated with decreased senescence in neural cells.[15] Another study revealed that Hedgehog signalling is reduced in aged endometrial stem cells, and the introduction of exogenous Hedgehog signalling has demonstrated antisenescence effects by modulating SERPINB2.[16] However, its effect and mechanism in cancer remain unknown. The crosstalk between Notch and Hedgehog signalling pathways in cancer senescence is complex and context-dependent.[12] Crosstalk between these pathways can either synergistically enhance or antagonistically regulate senescence outcomes in cancer cells, influencing tumour behaviour and therapy responses.[12,17]

HER2-positive breast cancer is characterised by overexpression of the HER2 receptor, making it a target for trastuzumab, a monoclonal antibody that inhibits HER2 signalling.[18] However, resistance to trastuzumab remains a significant challenge, leading to disease recurrence and poor prognosis. Targeting senescence pathways is gaining traction as a strategy to overcome trastuzumab resistance.[19] Trastuzumab resistance in human epidermal growth factor receptor 2-positive cancers arises from several mechanisms. One key factor is alterations in the HER2 receptor, such as truncated forms like p95HER2, which lack the extracellular domain targeted by trastuzumab, rendering it ineffective.[20] Increased HER2 expression or amplification can also saturate the drug's capacity to block all receptors. Another factor is the activation of alternative signalling pathways like the PI3K/AKT/mTOR pathway, which is often driven by PIK3CA mutations, bypassing HER2 inhibition, whereas HER family receptors, such as HER3 or EGFR, can continue oncogenic signalling.[21] The dysregulation of downstream signalling, including PTEN loss, further enhances resistance by hyperactivating these pathways. Epigenetic changes and immune evasion also play roles, altering HER2 signalling and diminishing trastuzumab's efficacy by reducing antibody-dependent cell-mediated cytotoxicity (ADCC).[22] Additionally, upregulation of stemness pathways (e.g., Notch, Hedgehog, Wnt) and changes in cell surface integrins enable cancer cells to bypass HER2 reliance.[19] Finally, trastuzumab internalisation and degradation within lysosomes limit its availability over time.[23] Together, these mechanisms create a complex resistance landscape that requires novel therapeutic approaches.

Notch and Hedgehog signalling pathways contribute to trastuzumab resistance by promoting cell survival and proliferation. Understanding how these pathways interact with HER2 signalling in trastuzumab-resistant cells is crucial.[24] Inhibiting Notch and Hedgehog signalling has been shown to enhance the expression of senescence-associated markers, such as $p16^{INK4a}$ and $p21^{CIP1}$, leading to growth arrest and reduced tumorigenicity in resistant cell lines.[25] Combining senescence-inducing agents with trastuzumab may provide a novel therapeutic approach to overcome resistance in HER2-positive breast cancer. The original value of this study can be found in its investigation of how the combination of trastuzumab and cilengitide affects cellular senescence in HER2-positive breast cancer cells that have developed resistance to trastuzumab. Previous research has focused on how these treatments reduce stemness and epithelial-mesenchymal transition (EMT)[3,4], but their effects on cellular senescence have not yet been explored in detail. This study demonstrated that this combination therapy upregulates senescence markers in trastuzumab-resistant cells and inhibits the Notch and Hedgehog signalling pathways, both of which play crucial roles in cancer progression. The involvement of specific inhibitors such as Fli06 and GANT61 to further suppress these pathways was also examined, suggesting a potential therapeutic strategy for overcoming drug resistance. The findings of this study indicate that targeting senescence pathways could be an effective strategy for addressing trastuzumab resistance in HER2-positive breast cancer, providing new directions for combination therapies. This study provides new insights into the molecular mechanisms of drug resistance and highlights a novel approach that could improve therapeutic outcomes for patients.

MATERIALS AND METHODS

Cell Culture

HCC1954 (ATCC Cat#CRL2338) and SKBR3 (ATCC Cat#HTB30) are well-known HER2-positive breast cancer cell lines, particularly recognised for their capacity to develop resistance to trastuzumab. These cell lines were cultured in DMEM supplemented with 10% FBS, 1% sodium pyruvate, and 2 mM L-glutamine. To induce trastuzumab resistance in HCC1954 and SKBR3, the cells were subjected to a gradual increase in trastuzumab concentrations (0.1–10 μM) over a period of three months. The development of resistance in these newly established cell lines was confirmed through MTT viability as-

says, with IC_{50} values calculated as described in our previous publication.[3] Chronic exposure to trastuzumab led to a marked increase in resistance, as evidenced by elevated IC_{50} values. The IC_{50} of SKBR3 cells exhibited a marked increase from approximately 0.2 µM to 2.6 µM, whereas that of HCC1954 cells, the IC_{50} dose from approximately 0.3 µM to 2.4 µM. The IC_{50} values for cilengitide were previously determined to be 0.8 µM for SKBR3-P, 0.6 µM for SKBR3-R, 0.6 µM for HCC1954-P, and 0.7 µM for HCC1954-R, as reported in earlier studies.[3,4]

Quantitative Real-Time PCR

Total RNA was isolated from SKBR3-P, SKBR3-R, HCC1954-P, and HCC1954-R cells using a Qiagen RNeasy Kit according to the manufacturer's instructions. cDNA synthesis was performed using 1 µg of RNA and the iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad). PCR amplification was conducted with the iTaq Universal SYBR Green One-Step Kit (Bio-Rad), with cycle threshold (Ct) values recorded on the ABI 7500 Real-Time PCR System (Applied Biosystems) and analysed using 7500 Software v1. The PCR protocol included an initial denaturation at 95 °C for 10 s, followed by 45 cycles of 95 °C for 10 s and 60 °C for 1 min. A subsequent melting curve analysis was carried out at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s.

Real-time PCR primers were designed using the Primer3 programme and synthesised by Macrogen (sequences provided in Table 1). Each PCR reaction was performed in triplicate for each sample and primer set, and technical replicates were included. The entire experiment was independently repeated three times using different biological samples. The RT-qPCR data were normalised to the housekeeping gene GAPDH. The ΔCt value was calculated as ΔCt = Ct (target gene) Ct (housekeeping gene). Fold changes were determined using the $2^{-\Delta Ct}$ method, and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ formula, where $2^{-\Delta Ct}$ (sample) was divided by $2^{-\Delta Ct}$ (control). The real-time PCR primers used in these experiments are listed in Table 1.

Table 1. Real-time PCR primers used in experiments.

GENE	SEQUENCE	Product length
AIF	F: GGCTGGATGAGATCAACAAGC	247
	R: TCAGGGTAGCTGAACGTCTC	
CCL3	F: TGTCCTCCTCTGCACCATG	173
	R: TGGTTAGGAAGATGACACCGG	
CST3	F: GTCGGCGAGTACAACAAAGC	194
	R: GCTTTCCTTTTCAGATGTGGC	
CSTA	F: CGCCACTCCAGAAATCCAGG	208
	R: CAAGTCCTCATTTTGTCCGGG	
CTTS	F: TGGGAGACATGACCAGTGAAG	153
	R: TCACTTCAGTAACACACCCTTTC	
CXLC8	F: TCTGTGTGAAGGTGCAGTTTTG	150
	R: ACAATAATTTCTGTGTTGGCGC	
FCER1G	F: TGGTCTTGCTCTTACTCCTTTTG	216
	R: CGTAAGTCTCCTGGTTCCTG	
FCN1	F: AGGTGTCATTGGAGAGAGAGG	175
	R: CCGGTCTAGCAGGTCCTTG	
G0S2	F: GATGGTGAAGCTGTACGTGC	193
	R: CTGCTTGCCTTTCTCCTGC	
LST1	F: CGAAGAGTAAAGAGGCTGGAG	184
	R: TGGGTTTGTTCTCAGCAATGC	
LYZ	F: AAGGTGTGAGTTGGCCAGAAC	241
	R: CAAAGCACTGCAGGATAAATGAC	
PSAP	F: TATGCTGAAGGACAATGCCAC	162
	R: CCAGGACGGCTCATTTCTCC	
S100A8	F: AATTTCCATGCCGTCTACAGG	205
	R: CTTTGTGGCTTTCTTCATGGC	
S100A9	F: ACCAATACTCTGTGAAGCTGG	230
	R: CTCGTGCATCTTCTCGTGG	
S100A11	F: ATCGAGTCCCTGATTGCTGTC	164
	R: TCCAGTTTCTTCATCATGCGG	
S100A12	F: AATACTCAGTTCGGAAGGGGC	214
	R: TGGTAATGGGCAGCCTTCAG	
SAT1	F: ATACTGCGGCTGATCAAGGAG	212
	R: TTGCCAATCCACGGGTCATAG	
SERPINA1	F: CCACGATATCATCACCAAGTTCC	202
	R: CTTATGCACGGCCTTGGAGAG	
TRYOBP	F: TAAGTGGTCTCCGTCCTGTCC	200
	R: AGTGATACGCTGTTTCCGGG	
NEAT1	F: GGCACAAGTTTCACAGGCCTACATGGG	205
	R: GCCAGAGCTGTCCGCCCAGCGAAG	

Heatmap Generation

Heatmaps were created using Python with the NumPy, Matplotlib, and Seaborn libraries. Python code was run in the Google Colab environment to produce the heatmap visualisation. Initially, the data—consisting of gene names (row labels), cell line names (column labels), and their associated quantitative values—was organised into a NumPy array. The data matrix was then visualised with the Seaborn library's heatmap function, utilising a diverging colour palette to differentiate between positive and negative values. The axes were appropriately labelled, and the heatmap was generated using Matplotlib.

Statistical Analysis

Statistical analysis was conducted with precision using two-way analysis of variance (ANOVA) to assess the interactions between the experimental variables. This was followed by Tukey's post hoc test to identify specific pairwise differences among the groups. Statistical significance was defined as follows: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$. The error bars in the figures represent the mean ± standard deviation (SD), calculated from three independent experiments, each conducted in triplicate, ensuring the robustness and reproducibility of the data.

RESULTS

Trastuzumab Plus Cilengitide Combination Increases Senescence in Markers

To analyse the effect of this combination on senescence, senescence-responsive gene expression was assessed using real-time PCR in trastuzumab-resistant SKBR3 and HCC1954 cells. A panel of 20 established genes associated with human and cellular ageing was employed, including *S100A9, CXCL8, CST3, TYROBP, LST1, FCN1, FCER1G, LYZ, CCL3, S100A8, CTSS, AIF1, S100A12, SAT1, G0S2, S100A11, PSAP, NEAT1, CSTA*, and *SERPINA1* (Figure 1).

In both cell lines, combination treatment led to significant upregulation of senescence markers, with strong statistical significance. No changes were detected in the expression of dimethyle sulfoxide (DMSO) or trastuzumab alone. Although cilengitide monotherapy-induced moderate upregulation, the combination of cilengitide and trastuzumab consistently resulted in the highest levels of marker expression. These results suggest that this combination treatment exerts a synergistic effect, enhancing the upregulation of senescence markers in both SKBR3 and HCC1954 cell lines.

Trastuzumab Plus Cilengitide Combination Decreases Notch and Hedgehog-Responsive Gene Expressions

Previously, an increase in Notch and Hedgehog pathways in HER2-positive cells was demonstrated. It was also shown that Notch signalling is reduced by the combination of trastuzumab and cilengitide although its impact on the Hedgehog pathway has not been established. Given the critical crosstalk between these pathways, the effects of trastuzumab and cilengitide were assessed both individually and in combination. The results revealed that cilengitide, either alone or in combination with trastuzumab, significantly decreased Notch and Hedgehog signalling responses in SKBR3 and HCC1954–resistant cells (Figure 2). No alterations in signalling were observed with DMSO or trastuzumab alone. Additionally, cilengitide monotherapy was less effective than combination treatment in inhibiting Notch and Hedgehog signalling in both cell lines.

Combined Inhibition of Notch and Hedgehog Signalling Induce Senescence

Given the observed reduction in Notch and Hedgehog signalling pathways from the combination of trastuzumab and cilengitide, Notch and Hedgehog inhibitors were employed to investigate whether similar effects on senescence markers could be achieved. First, the effects of Notch and Hedgehog inhibitors on SKBR3 and HCC1954-resistant cells were analysed using different concentrations (0.01, 0.1, and 1 μM). To assess Notch pathway inhibition, the expression levels of *Hes, Hey, Gata3,* and *Ptcra* genes were evaluated. For Hedgehog pathway inhibition, the expression levels of *Gli1, Gli2, Hhip, Ptch1*, and *Ptch2*

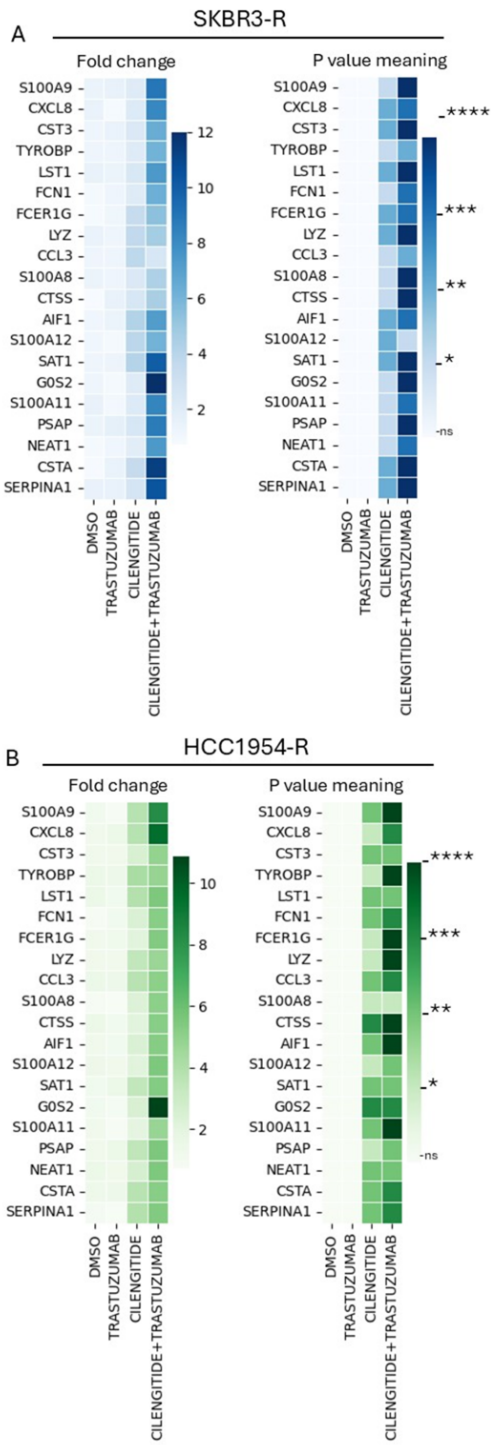

Figure 1. Senescence-responsive gene expression induced by cilengitide + trastuzumab combination therapy in HER2-positive trastuzumab-resistant cell lines. Resistance to DMSO, trastuzumab, and cilengitide monotherapy and cilengitide+trastuzumab combination therapy in (A) SKBR3 and (B) HCC1954 cells. Statistical analysis was performed using a two-way ANOVA variation test and Tukey's post hoc test to determine significance. Differences were considered significant as non-significant (ns) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, n = 3.

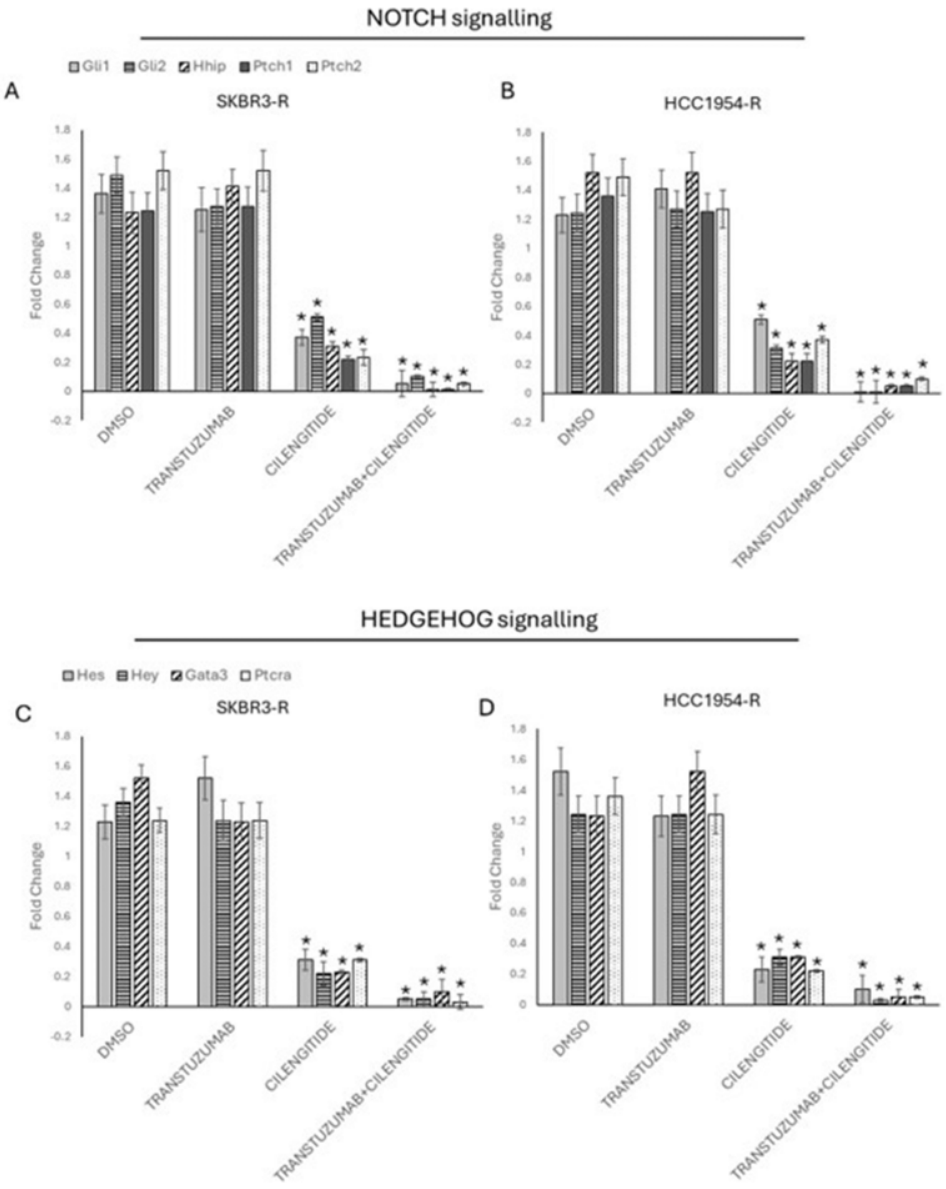

Figure 2. Trastuzumab + celangitide combination decreases NOTCH and HEDGEHOG responsive gene expressions. Cilengitide monotherapy and trastuzumab + cengitide combination decrease NOTCH-responsive gene expression in A) SKBR3-R, B) HCC1954-R cells, and Hedgehog-responsive expression in C) SKBR3-R, D) HCC1954-R cells. R: resistance. Statistical analysis was performed using a two-way ANOVA variation test and Tukey's post hoc test to show significance * $p \leq 0.05$, n=3.

genes were analysed (Figure 3). 1 µM of Fli06 and GANT61 showed significant decreases in both pathways and were used in further experiments. This study elucidated whether cilengitide and trastuzumab induce senescence markers via the Notch and Hedgehog pathways. To this end, the Notch inhibitor Fli06 and the Hedgehog inhibitor GANT61 were administered individually or in combination. RNA was collected 6 h following incubation with 1 µM Fli06 and GANT61 (Doses were determined based on their effects on the pathways as shown Figures 3A-D). While individual inhibition of Notch and Hedgehog pathways resulted in moderate increases in senescence markers, combined inhibition of both pathways led to a more pronounced up-regulation of these markers in SKBR3- and HCC1954-resistant cells (Figure 4). DMSO-treated resistant cells were used as a control for comparison.

DISCUSSION

Senescence, characterised by irreversible cell growth arrest, plays a pivotal role in cancer biology by suppressing tumours and influencing their response to treatment.[26] Previous research has demonstrated that combining trastuzumab with cilengitide significantly reduces stemness and EMT in both trastuzumab-resistant and -sensitive HER2-positive cell lines.[4]

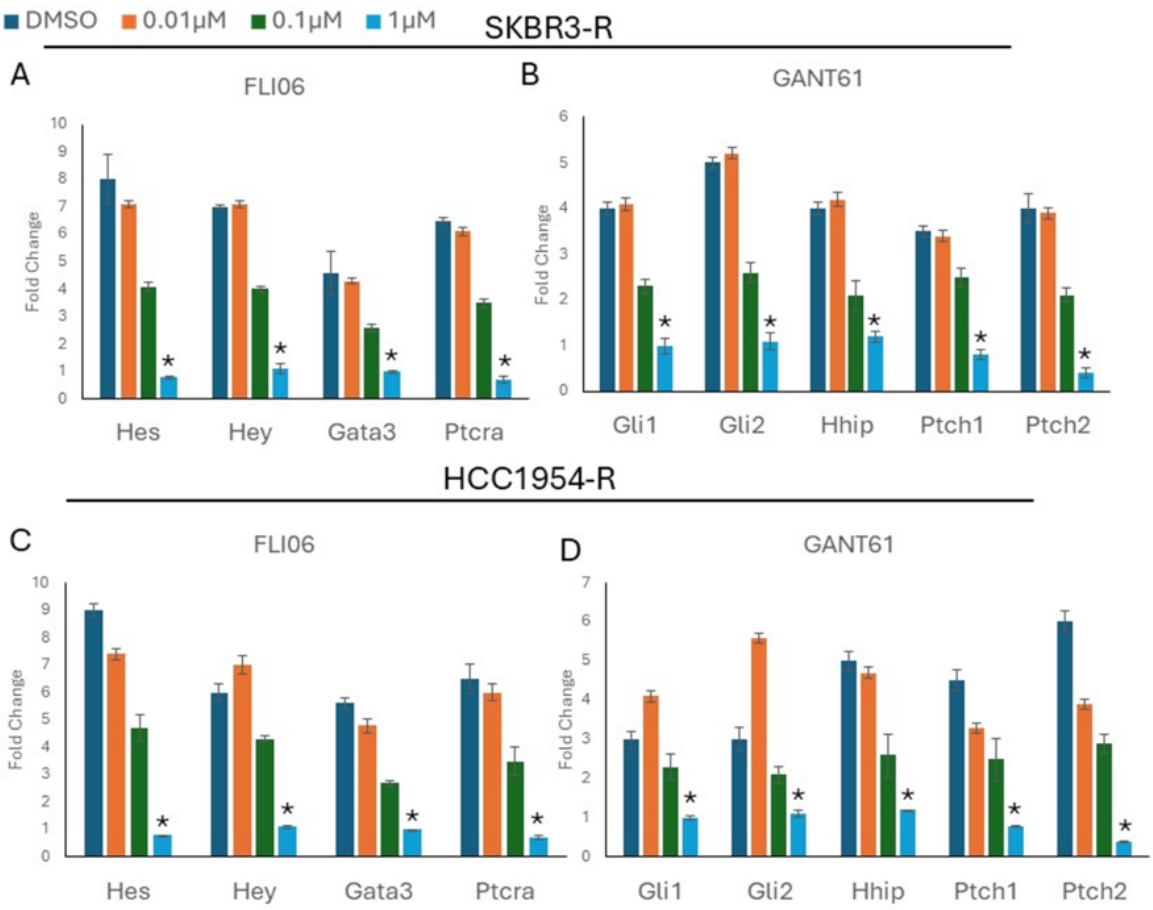

Figure 3. FLI06 decreased the Notch pathway and GANT61 decreased the Hedgehog pathway dose dependently. Notch responsive gene expressions analysed in the presence of FLI06 in a dose-dependent manner (A) in SKBR3-R, C) HCC1954-R. B) Hedgehog responsive gene expressions analysed in the presence of GANT61 in a dose-dependent manner (B) in SKBR3-R and D) HCC1954-R cells. R: resistance. Statistical analysis was performed using one-way ANOVA variation test and Tukey's post hoc test to show significance * $p \leq 0.05$, n=3.

However, the impact of this combination on senescence has not been explored. In this study, the combination of trastuzumab and cilengitide significantly upregulated senescence markers in trastuzumab-resistant HER2-positive cell lines. This finding aligns with research suggesting that targeting senescence pathways could be a novel approach to overcome cancer resistance. It has been shown that senescence can suppress tumour progression by halting the growth of resistant cells. For instance, senescence-associated secretory phenotypes (SASP) can alter the tumour microenvironment, thereby making it less favourable for tumour growth.[27]

The effects of trastuzumab and cilengitide combination on senescence were more pronounced compared with either treatment alone, suggesting a synergistic interaction between trastuzumab and cilengitide in promoting senescence. These findings underscore a promising new approach to overcoming trastuzumab resistance in HER2-positive breast cancer by inducing cellular senescence. Cilengitide, known to influence various cellular processes such as survival, proliferation, and senescence, has been the subject of debate regarding its effects on senescence.[27] Studies have reported both pro- and antisenescent effects depending on the cellular context and specific integrins . This study contributes to the literature by demonstrating that cilengitide, an RGD-binding integrin inhibitor, can induce senescence in HER2-positive breast cancer cells. This finding agrees with other studies showing that CWHM12, another RGD-mimicking integrin blocker, enhances senescence in hepatic stellate cells.[7-10]

Further investigation into the underlying signalling pathways revealed that the combination of trastuzumab and cilengitide significantly reduces Notch and Hedgehog signalling responses in resistant cell lines.[3,4] These pathways are critical for development, tissue homeostasis, and cancer progression. The results suggest that the upregulation of senescence markers in

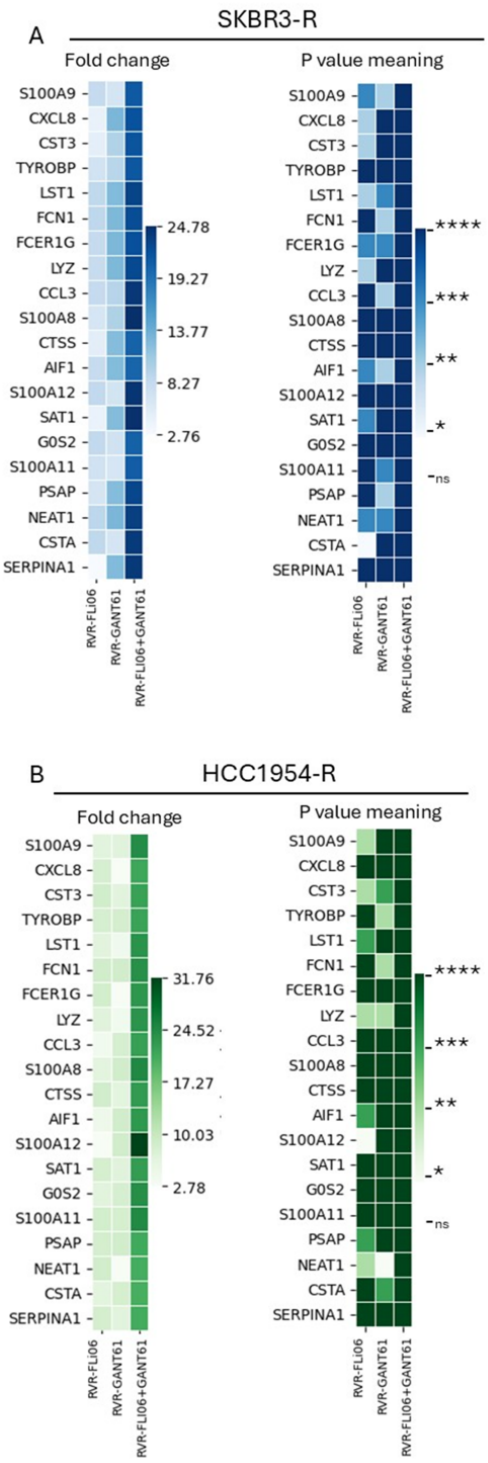

Figure 4. Senescence-responsive gene expression induced by the combined inhibition of the NOTCH and HEDGEHOG pathways in HER2-positive trastuzumab-resistant cell lines. Resistant cells compared with resistant cells treated with Fli06, GANT61, or Fli06+GANT61 in (A) SKBR3 and (B) HCC1954 cells. Statistical analysis was performed using a two-way ANOVA variation test and Tukey's post hoc test to determine significance. Differences were considered significant as non-significant (ns) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, n = 3.

trastuzumab-resistant cell lines is achieved through the inhibition of both Notch and Hedgehog pathways, either through the combination of trastuzumab and cilengitide or through specific inhibitors (Fli06 for Notch and GANT61 for Hedgehog). This indicates that the pro-senescent effects of the trastuzumab-cilengitide combination are, at least in part, mediated by inhibiting these pathways. This study provides new insights into how targeting these pathways can overcome trastuzumab resistance, highlighting the complex and context-dependent crosstalk between Notch and Hedgehog signalling in cancer senescence. These findings reinforce the expanding body of evidence that targeting senescence pathways is a promising strategy for overcoming trastuzumab resistance in HER2-positive breast cancer. The results clearly demonstrate that the inhibition of Notch and Hedgehog signalling pathways can drive cancer cells towards senescence, which acts as a barrier to tumour progression. Previous research supports this, indicating that the modulation of Notch signalling can either promote or suppress senescence, depending on the cancer type and context.[28,29] For instance, studies have shown that Notch1 and Notch2 upregulate p21 and p16, key regulators of senescence.[29]

Similarly, Hedgehog signalling, which maintains stem cell properties in cancer cells, plays a vital role in treatment resistance. By inhibiting this pathway with agents like GANT61, cancer cells become more sensitive to therapy, as shown in glioblastoma models.[10,17] The synergistic effect observed with the combination of trastuzumab and cilengitide, as outlined in this study, further supports the idea that integrating senescence-inducing agents into treatment regimens can significantly improve therapeutic outcomes in trastuzumab-resistant cancers.[2,19] Furthermore, the findings align with prior research indicating that integrin inhibitors like cilengitide, which target RGD-binding integrins, disrupt cancer cell adhesion and migration, thereby enhancing the effects of other therapies like trastuzumab.[1,4] This study provides compelling evidence for the clinical potential of combining trastuzumab with agents that target senescence pathways, providing a robust approach to improving treatment efficacy in patients with HER2-positive breast cancer who are resistant to current therapies.

CONCLUSION

In conclusion, the combination of trastuzumab and cilengitide induces senescence in HER2-positive trastuzumab-resistant cell lines and may be inhibited through Notch and Hedgehog signalling pathways. The data presented, not only highlight the potential of combining trastuzumab with cilengitide and underscore the need for further investigation into the crosstalk between Notch and Hedgehog signalling in the context of resistance. Ultimately, targeting senescence pathways is a compelling strategy for improving therapeutic outcomes in HER2-positive breast cancer, particularly in the setting of trastuzumab resistance. This approach could pave the way for more effective

combination therapies that enhance the durability of responses in cancer treatment.

These findings provide a promising new strategy for overcoming trastuzumab resistance in HER2-positive breast cancer and highlight the potential of senescence-inducing therapies as part of a comprehensive treatment approach. Further studies are warranted to explore the clinical implications of these findings and identify additional targets within the senescence pathways that could enhance the efficacy of current therapies.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- I.E., A.B.B.E.; Data Acquisition- I.E., A.B.B.E.; Data Analysis/Interpretation- I.E., A.B.B.E.; Drafting Manuscript- I.E., A.B.B.E.; Critical Revision of Manuscript- I.E., A.B.B.E.; Final Approval and Accountability- I.E., A.B.B.E.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

ORCID IDs of the Authors

Asiye Busra Boz Er 0000-0002-1275-4470
Idris Er 0000-0003-2139-1787

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How to cite this article

Boz Er AB, Er I. Notch and Hedgehog Signalling Axis Drive Senescence in HER2-Positive Breast Cancer Resistant to Trastuzumab. Eur J Biol 2024; 83(2): 213–221. DOI:10.26650/EurJBiol.2024.1531120