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Short Communication

NF-kB oscillation profiles decode response to anti-EGFR monoclonal antibodies

Donatella Romaniello [a], Lorenzo Dall'Olio [b], Martina Mazzeschi [c], Anna Francia [a], Federica Pagano [a], Valerio Gelfo [a], Gabriele D'Uva [a,c], Enrico Giampieri [a,1], Mattia Lauriola [a,c,1,*]

[a] Department of Medical and Surgical Sciences, University of Bologna, via Massarenti 9, 40138 Bologna, Italy
[b] Laboratory of Data Science and Bioinformatics, IRCSS Institute of Neurological Sciences, Bellaria Hospital, via Altura 3, 40139 Bologna, Italy
[c] IRCCS Azienda Ospedaliero-Universitaria di Bologna, via Massarenti 9, 40138 Bologna, Italy

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ABSTRACT

A direct connection between an inflammatory environment and cancer has been extensively proven over the years. We previously reported that the presence of interleukin 1 (IL-1) is responsible for the lack of response to monoclonal antibody targeting epidermal growth factor receptor (EGFR) in colorectal cancer (CRC).

Considering the driver role of NF-kB in controlling the expression of IL-1, herein, we investigate the dynamics of the oscillatory profile of the NF-kB response to monoclonal antibody, on the background of an inflammatory environment. NF-kB is a typical transcription factor that displays intrinsic oscillatory behavior, whose biological relevance in term for example of decoding response to monoclonal antibodies, remains unclear.

Using live cell luciferase techniques, we recorded NF-kB activity over time in response to cetuximab (CTX) alone or in combination with IL-1 cytokines. Our results revealed an additive effect of these two agents on NF-kB activation, which was specific to CTX responsive cells. In contrast, CTX resistant cells did not display a significant change in the NF-kB profile under the IL-1 plus CTX combination. These results suggest an immediate interactive crosstalk between IL-1 and EGFR in the activation of NF-kB signaling pathway, which may lay the basis for the development of drug tolerant persister cells (DTP), leading to CTX resistance.

1. Introduction

NF-kB acts as a versatile mediator of gene expression, with its localization in the nucleus or cytoplasm primarily influenced by the microenvironment, thus prompting a wide range of cellular responses.

In unstimulated cells, NF-kB displays a cytosolic localization, and it shifts to a nuclear localization and DNA-binding in the presence of stimulating agents, such as proinflammatory cytokines (IL-1, TNF-α), growth factors, lipopolysaccharide (LPS), viral double-stranded RNA, cellular stress (induced by UV, ionizing radiation and reactive oxygen species) [1].

The shuttling of NF-kB between the nucleus and cytoplasm along with the oscillatory profile, is facilitated by the activation and degradation of the inhibitor subunit, named Inhibitory-kB (IkB). The NF-kB oscillatory dynamics are instrumental to segmenting time and provide a temporal window of specific patterns of controlled gene expression [2].

Indeed, by controlling the DNA-binding activity and subcellular localization of NF-kB, IkB plays a crucial role in regulating the transcription of NF-kB target genes [2]. In details, IkB proteins anchor NF-kB to the cytoplasm, and upon phosphorylation undergo degradation, thus NF-kB dimers are free to translocate into the nucleus to regulate expression of target genes [3,4].

Induction of NF-kB activity does not require protein synthesis. This discovery has inspired a field of research, aimed at uncovering the pathways that enable specific signals to trigger the activity of nuclear transcription factors (TFs). By shedding light on these mechanisms, we can gain a better understanding of how cells respond to various stimuli and develop new approaches to treat diseases associated with NF-kB dysregulation [5].

* Corresponding author.
E-mail address: mattia.lauriola2@unibo.it (M. Lauriola).
[1] Equally contributed.

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As an important TF and mediator of inflammatory response, any deregulation of NF-kB signaling, due to genetic mutation or microenvironment cues, leads to a pro-tumorigenic response inducing abnormal cell proliferation and differentiation, angiogenesis, enhanced metastasis, and even resistance to treatments [6]. The fundamental role of NF-kB in cancer initiation and progression has been recognized in several types of cancers [7–10], including colorectal cancer (CRC) [10, 11]. A significant correlation between expression levels of NF-kB and K-RAS status has been described in human colorectal adenocarcinoma [12,13].

In patients with K-RAS mutations, the activity of NF-kB signaling is typically elevated compared to those with wild-type K-RAS. This increase in signaling has been associated with a pro-tumorigenic response, including enhanced chemotherapy tolerance and decreased survival rates [14]. Several clinical studies confirmed that adding the anti-EGFR tyrosine kinase inhibitor, gefitinib, in combination with FOLFOX-4 regimen failed to improve efficacy in patients with activated NF-kB signaling [15].

In line, in our previous work, we demonstrated that CRC cells resistant to EGFR-targeted antibody CTX displayed elevated tumor derived IL-1 production, both alpha and beta [16]. Moreover, in a cohort of 150 CRC xenopatients, we found a positive association between high levels of IL1A, IL1B and IL-8 cytokines and poor response to CTX [17].

These results suggest the involvement of NF-kB in the response to anti-EGFR targeted therapy, but a clear and in real time dynamic definition on how monoclonal antibodies anti-EGFR affect the activation profile of NF-kB is still lacking.

Here we employed a luciferase-p65 reporter knock-in, to follow NF-kB activation profiles in both CTX sensitive and resistant cell lines. We imposed CTX stimulus alone or in combination with IL-1 and recorded the NF-kB oscillatory behavior over time.

We unveiled a peculiar NF-kB activation profile, which appears independent on CTX stimulus alone, indeed no changes were recorded under CTX stimulus. But interestingly, when IL-1 cytokines were combined with CTX, an additive effect on NF-kB activation was recorded upon CTX administration. These results suggest an interactive crosstalk between IL-1/EGFR/NF-kB in the temporal regulation of NF-kB, which may result in the insurgence of resistance to anti EGFR monoclonal antibody in metastatic CRC.

By unraveling the complex interplay between these pathways, we aim to identify new therapeutic combinations for improving the effectiveness of anti-EGFR monoclonal antibody therapy in patients with metastatic CRC.

2. Material and methods

2.1. Cells lines

Caco-2 Parental NF-kB and Caco-2 CXR NF-kB (Cetuximab Resistant Cells) were transduced with the Signal Lenti Reporter Assay (QIAGEN), with a pseudotyped lentiviral particles combined with specific NF-kB binding site, basic promoter elements and firefly luciferase as reporter gene. Both cell lines were maintained at 37 °C 5 % CO2 in DMEM supplemented with 10 % of FBS (fetal bovine serum), 1 % Pen/Strep and 1 % l-Glu. Puromycin 5 μg/mL was added to the medium for both cell lines. Caco-2 CXR NF-kB were maintained in medium supplemented with cetuximab (10 μg/mL - Erbitux, Merck KgaA, Gemany).

2.2. AlamarBlue assay

In a 96-well plate 2000 cells/well were seeded in 100 μL of DMEM 10 % FBS. The day following the seeding, the first quantification was performed (Time 0, T0) after 4 hrs of incubation with AlamarBlue (40 μM), measuring the fluorescence using VICTOR2™ 1420 multilabel counter (Perkin Elmer, Massachusetts, USA), at a wavelength of 595 nm. When the T0 measurement was taken, the medium was changed, and treatments were added to the plates. After 96 hrs, proliferation was detected with AlamarBlue following the same procedure. In each plate, some wells were left as background, with just medium and treatments, and used in the analysis of the data. Each experiment was repeated at least three times.

2.3. Luciferase assay

For the Luciferase assay 3000 cells/well of Caco-2 Parental NF-kB and Caco-2 CXR NF-kB were seeded in 96-well flat clear bottom white polystyrene TC-treated microplates, for improving luminescent signal and reduce background (Corning #3610) in 150 uL of medium. The following day, AlamarBlue assay at T0 was performed as described previously. Thereafter, medium was changed and Luciferin (D-Luciferin, Gold Biotechnology, St. Louis, MO) was added (100 μg/mL) along with treatments. The first fluorescence signal was measured after 30 mins of incubation (Time 0) followed by a kinetic loop of luminescence detections every 20 min for a total of 180 cycles. This detection module was repeated for at least 60 hrs, thus allowing to obtain several acquisitions for each well. Bioluminescence was measured using SPARK multimode microplate reader (Tecan Group Ltd., Männedorf, Switzerland), with the following specifications: Mode: luminescence, Wavelength: 560 nm, Attenuation: none, Settle time (ms): 0, Integration time (ms): 1000, Output: counts/s, Temperature: 37 °C, CO2: 5 %, O2: 20 %. The resulting data were plotted as "NF-kB luciferase activity (Arbitrary Units)" on the y-axis. Some wells with only medium and treatments were used as background and employed for data analysis. Data from the Luciferase assay were normalized with data from the proliferation assay (AlamarBlue assay).

2.4. Colony forming assay

The colony-forming assay was used to compare the degree of transformation of engineered Caco-2 Parental NF-kB and Caco-2 CXR NF-kB cells according to their ability to grow at a reduced density. One thousand cells were seeded in 12-well plates with and without CTX (10 μg/mL). The following day treatments were added, as reported in the figure legends. After eight-ten days, the medium was removed, and the cells were washed with PBS and fixed with 4 % paraformaldehyde for 20 mins at room temperature. After washing with PBS, the cells were stained with crystal violet 0.05 % for 30 mins, then washed with water to remove any excess of dye. Thereafter, crystal violet was dissolved in 10 % acetic acid and each solution was measured at a wavelength of 595 nm using VICTOR2™ 1420 multilabel counter (Perkin Elmer, Massachusetts, USA), Absorbance data collected were analyzed through Prism software and showed as a column chart.

2.5. Data preprocessing

Data preprocessing and data analysis were performed on a common laptop, using Python 3.8.16 together with some basic libraries included in the version 3 of the Anaconda package [18]. We first averaged the signals coming from empty sample wells, to obtain the average background noise. Then we subtracted such average noise from every signal to obtain the denoised version of each signal. Please note, that, Caco-2 Parental NF-kB and Caco-2 CXR NF-kB cell lines were acquired in two different sampling tanks, the background averaging procedure was performed separately for each cell lineage, therefore having one average background noise to subtract from CXR cells, and another background noise signal to subtract from Parental cells. In order to make further signals comparisons meaningful, we needed different signals on a comparable scale, therefore we performed a log scaling. This step was accomplished by dividing the value by geometric mean, which mathematically corresponds to the arithmetic mean of the log scaled values. The last step of the preprocessing was the detrending step, eliminating high-period components from each signal by subtracting its respective

trend. This step was performed with a locally weighted scatterplot smoothing (LOWESS) algorithm, using 25 % of time points for the moving window width [19]. This algorithm consists of a non-parametric local linear regression, which is capable to capture non-linear trends. For consistency, we tried to vary the moving window width from 10 % up to 40 % of points, observing no clear difference over the obtained results.

2.6. Data analysis

At the start of the analysis step, each signal has been denoised, logscaled, and detrended. Then each signal undergoes the computation of its autocorrelation function, which consists in calculating the correlation of a signal with its shifted copy, repeating for every possible value of the time-lag (from 0 up to the signal time duration). This procedure helps identifying the presence of periodic patterns within the signal, that could otherwise be hided, even partially, by noise. This function gives insights into how informative the signal is for predicting its value after a given time lag, giving us information regarding periodic fluctuations within the signal. The last step was to perform a fast Fourier transform (FFT) of each autocorrelation function. This step is commonly used to decompose a signal into its different frequency/period components. By doing so we could investigate each autocorrelation dominating period components and compare different signals autocorrelation Fourier spectra. This last step's results were consistent when varying the LOWESS smoothing parameter within the interval of 10∼50 % proving that this procedure was robust and that results are not significantly affected by preprocessing-related artifacts.

Since for each combination of cell lineage (CXR or Parental) and condition (Control, CTX, IL-1, CTX+IL-1) we had 5 different signals acquired at the same time, we ultimately performed an average over these corresponding 5 signals, reducing the number of signals from 40 down to 8 average signals. In this way, we ensured that the outcome was consistent across similar signals, and not due to strong noise fluctuations

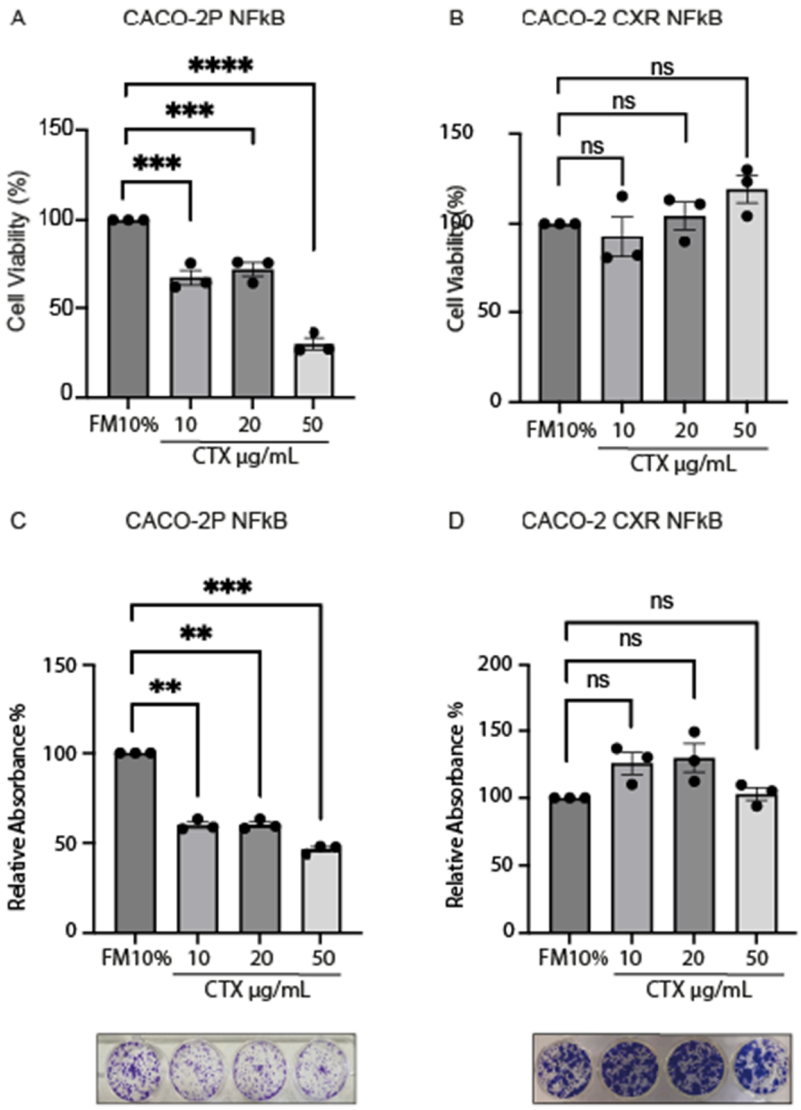

Fig. 1. Response to cetuximab treatment in sensitive and resistant cell line. Alamar blue assay was performed to assess cell viability. 2×10^3 cells were seeded in 96-well plates. The next day, Alamar blue was added to detect T0 signal. Thereafter, media was changed to contain the following treatments: DMEM full medium, 10 % FBS (FM10 % and increasing concentration of cetuximab (CTX) 10, 20, and 50 µg/mL. The fluorescent signal was detected after 4 days in Caco-2 P_ NF-kB (A) and Caco-2 CXR_ NF-kB (B). The data represent the averages ± SEM of three technical replicates from a single experiment, which has been repeated twice (the repeat is shown in supplementary figure 1A-B). A colony forming assay was carried out by seeding 1×10^3 cells in 12-well plates. The following day, treatments with increasing doses of CTX were added. Cell survival was determined as relative absorbance (%) in P_NF-kB (C) and CXR_ NF-kB (D) cell lines. The data represent the averages ± SEM of three technical replicates from a single experiment, which has been repeated twice (the repeat is shown in supplementary figure 1C-D). Statistical significance was assessed using one-way ANOVA, Dunnett's multiple comparisons test (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

occurring in only one of them. This procedure was applied to: pre-processed signals, autocorrelation functions, and Fourier period spectra, computing in every case the average signal, and the standard error, for every given time point or period.

3. Results

3.1. Establishment of the CTX-sensitive and resistant NF-kB luciferase cellular models

In order to investigate the molecular mechanisms implicating NF-kB in the emergence of resistance to the monoclonal antibody (moAb) CTX, we employed Caco-2 parental cell line sensitive (Caco-2 P) and resistant to CTX (Caco-2 CXR), recently established in our laboratory, and engineered with pseudo-typed lentiviral particles, combined with specific NF-kB binding site, basic promoter elements and firefly luciferase as a reporter gene (Caco-2P NF-kB and Caco-2 CXR NF-kB respectively) [17]. Caco-2P NF-kB and Caco-2 CXR NF-kB, hereafter called P_NF-kB and CXR_NF-kB, were tested for CTX (10, 20, 50 μg/mL) responsiveness by proliferation assay. Parental cells displayed an immediate response to CTX, showing a significant decrease in proliferation when compared to untreated conditions, as reported in Fig. 1A and Supplementary Figure 1A. On the other hand, CXR_NF-kB confirmed the addiction to the drug, by growing undisturbed, compared to untreated cells (Fig. 1B and

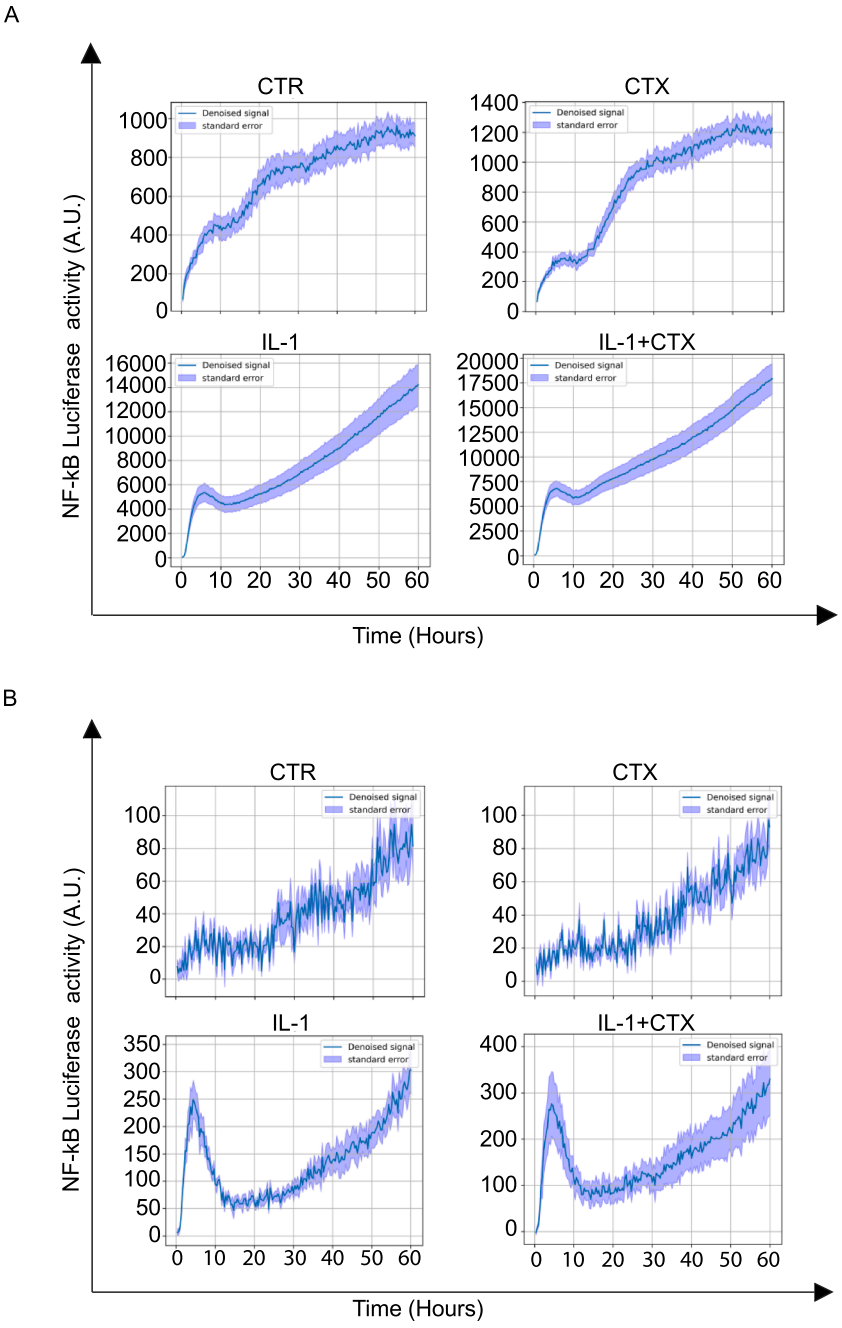

Fig. 2. In concomitance with cell viability, luminescence signal increases over time, but CXR_NF-kB has a lower basal luciferase signal. Cells (3×10^3 cells/well) were seeded in 96 well-plates. The day after, alamar blue was added to measure cell viability at T0. Thereafter, medium containing luciferin was changed and treatments were added, control medium (CTR, 10 %FBS), CTX (10 μg/mL), IL-1α/β (10 ng/mL) alone and in combination with CTX. Luminescence signal was measured every 20 min for 60 hrs total followed by alamar blue assay in P_NF-kB (A) and CXR_NF-kB (B). Each experiment was done with technical quintuplicates. The biological replicate is reported as Supplementary Figure 2.

Supplementary Figure 1B).

Next, we confirmed the acquisition of resistance to CTX in a long-term clonogenic assay, by following both P_NF-kB and CXR_NF-kB proliferation for ten days upon CTX treatment. NF-kB sensitive cells displayed a 40 % decrease in proliferation under 10 and 20 μg/mL of CTX treatment compared to the untreated condition and a >50 % decrease with the higher concentration of CTX 50 μg/mL, with a small number of drug-tolerant persistent cells (DTPs) (Fig. 1C and Supplementary Figure 1C), whereas CXR_NF-kB displayed no response to CTX, with a tendency toward the CTX addiction because cells were growing even better with the drug, thus confirming the acquisition of resistance (Fig. 1D and Supplementary Figure 1D).

3.1.1. *CTX resistant cells display greater NF-kB oscillation amplitude compared to sensitive cells*

Increased production of both IL-1 alpha and beta isoforms was associated to resistance to CTX in patients [16]. Considering that both growth factors and pro-inflammatory cytokines are important NF-kB

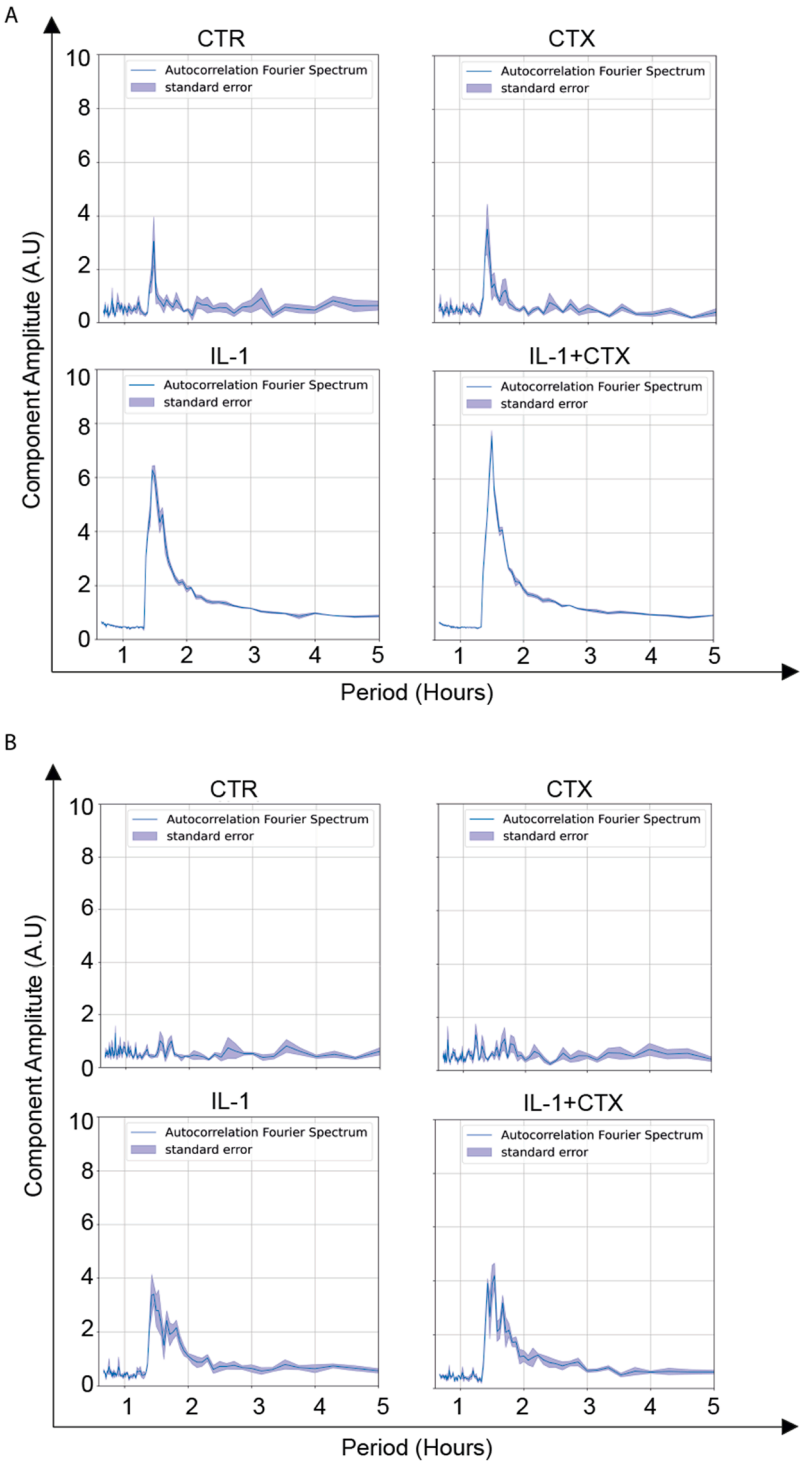

Fig. 3. CTX treatment in combination with IL-1, enhances the amplitude of NF-kB oscillatory period. Data acquired from the experiment and represented in Fig. 2 were processed showing the amplitude of NF-kB oscillation periods. The biological replicate is reported as Supplementary Figure 3.

activators, we further explored the NF-kB profile in both sensitive and CTX resistant cells. During the onset of the experiment lasting 60 hrs, the luciferase amplitude increased constantly, for both cell models, most likely due to cell proliferation. Indeed, in each well treated with vehicle (CTR), CTX, IL-1α/β and their combination, we identified a constant increase over time for NF-kB luciferase signals (Fig. 2 and Supplementary Figure 2). Of note, CTX resistant cells (Fig. 2B) showed a basal NF-kB activation 10-fold lower compared to sensitive cells (Fig. 2A), and most importantly, it was not affected by the CTX stimulus, thus suggesting the refractoriness to the drug (Fig. 2B).

3.1.2. *CTX treatment in presence of IL-1 enhances the amplitude of NF-kB oscillatory period in sensitive cells*

Since we were only interested in the NF-kB activity, the first step required a detrending operation in order to extract the fluctuating component of the signal, such as proliferation. Thus, we employed the LOWESS technique, a specific type of nonlinear regression system particularly useful for our purposes [19]. By subtracting the LOWESS of the signal, we obtained its detrended version, over which the autocorrelation was computed and we decomposed such autocorrelation into oscillatory components with different periods (hours). It was previously reported that the activity of NF-kB is regulated through negative feedback loops provided by the inhibitors IkB and A20, resulting in a period of about 90 min[4]. Our results confirmed this measurement, and sensitive

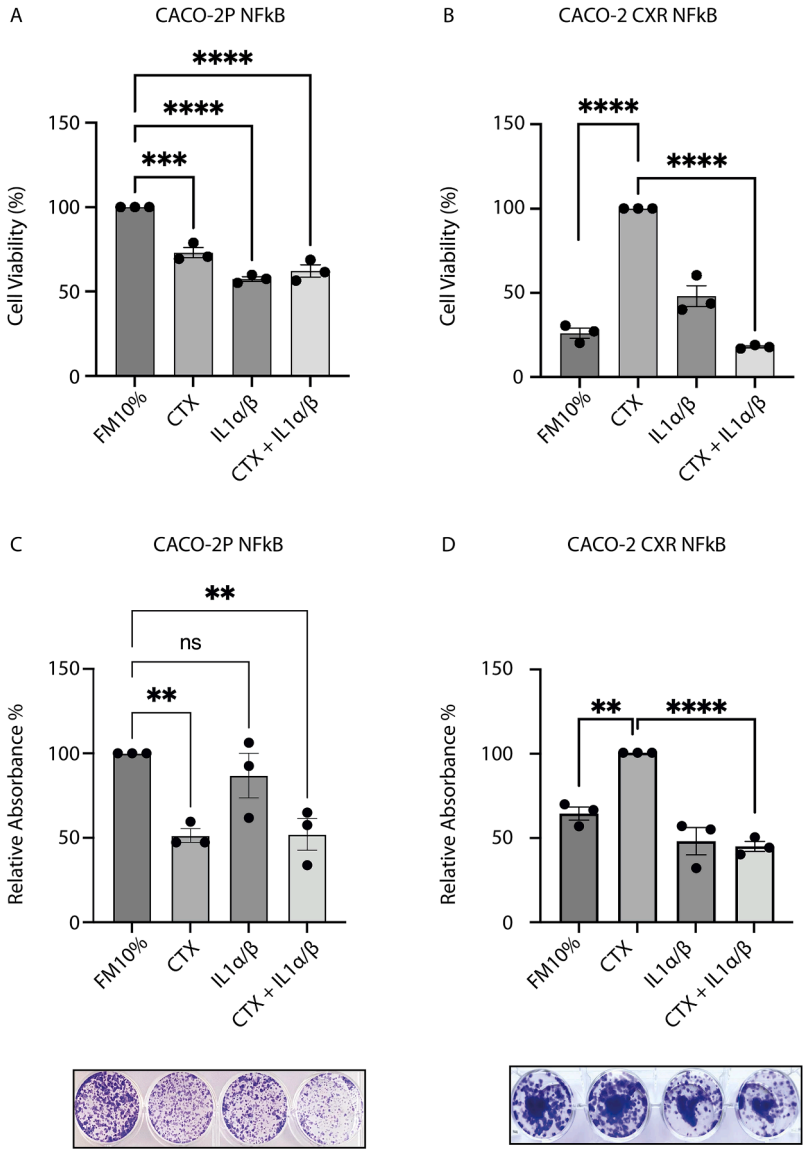

Fig. 4. Effect of IL1 and CTX stimulation in sensitive and resistant cells Cell viability was evaluated through Alamar blue assay. Cells (2×10^3) were seeded in 96-well plates and T0 fluorescent signal was detected the next day, after incubation for 4 hrs with Alamar blue. Thereafter, media was changed containing the following treatments: DMEM full medium 10 % FBS, (FM10 %), control media with Cetuximab 10 μg/mL (CTX), Interleukin IL1 α and Interleukin β, 10ng/mL total (IL1α/β) alone or in combination with CTX (CTX+ IL1α/β). Fluorescent signal was detected after 4 days. Graphs in A and B depict the averages ± SEM of three technical replicates from a single experiment that has repeated twice (shown in supplementary figure 4A-B). The statistical analysis was conducted on each biological replicates. Column chart shows one result of two independent experiments performed in triplicates in both P_ NF-kB (A) and CXR_ NF-kB (B) cell lines. For the colony-forming assay, 1×10^3 cells were seeded in 12-well plates. The following day, treatments were added, as described above in A and B and clonogenicity was followed for 10 days and represented as relative absorbance (%) in P_ NF-kB (C) and CXR_ NF-kB (D) cell lines. Graphs in C and D depict the averages ± SEM of three technical replicates from a single experiment that has repeated twice (shown in supplementary figure 4C-D). The statistical analysis was conducted on each biological replicates. Statistical significance was assessed using one-way ANOVA, Dunnett's multiple comparisons test (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$).

cells display a main peak of NF-kB activation with a period of about 90 min, which did not change upon CTX introduction [4].

IL-1 stimulus, as expected, was able to enhance the amplitude of NF-kB oscillation insisting on a period of 90 min. Notably, CTX stimulation when combined with IL-1, recorded an additive effect on the amplitude of NF-kB (Fig. 3A). On the other hand, CTX-resistant cells lack a visible and discrete peak for NF-kB. The introduction of IL-1 alone attempted to restore the 90 min period, but the amplitude was still modest and most importantly the addiction of CTX did not seem to affect this factor (Fig. 3B). In conclusion, sensitive cells maintain a defined and synchronized oscillatory profile compared to the CTX resistant cells, indeed the vast majority of the NF-kB oscillations occurred around a 90 min period. On the contrary, the introduction of IL-1 alone or in combination with CTX is responsible for the enhanced amplitude. Notably, resistant cells have a wider distribution, with NF-kB signals more subdued with minor fluctuations, which may suggest a more chaotic system, less prone to synchronization in the presence of stimuli. To sum up, CTX in combination with IL-1 causes NF-kB levels to fluctuate more significantly over each cycle, by enhancing the amplitude of the NF-kB oscillatory period.

3.1.3. Coupling NF-kB abundance with cell phenotypes: effect of the oscillatory pattern on cell proliferation

Next, we paired the distinct NF-kB activation profiles to the proliferation output. IL-1α and IL-1β cytokines besides playing a fundamental role in acute and chronic inflammation, show a pro-tumor effect, both by inhibiting the anti-tumor immune response and by stimulating cell proliferation, survival and invasiveness [20]. Additionally, IL-1α/IL-1β are well-known mediators of cell senescence via SASP, as we recently reported [21–24].

IL-1α and IL-1β stimulation had an impact on proliferation compared to untreated condition, which is the control condition, full medium with 10 % FBS (FM10 %). In P_NF-kB cells, we observed a reduction in cell viability under IL-1 or CTX stimuli and the same results were obtained when IL-1 and CTX were combined (Fig. 4A and Supplementary Figure 4A). On the contrary, resistant cells were growing undisturbed under CTX treatment, which represents the baseline condition, displaying a drug addiction. This dependency is evident, as the removal of CTX from the growth medium results in poor proliferation, as shown in Fig. 4B and D and previously reported [16]. While, there is an inhibition of proliferation with IL-1α/IL-1β, most likely because of the induction of cell senescence, as previously reported [22], the combination with CTX further enhances the inhibition of cell proliferation (Fig. 4B and Supplementary Figure 4B).

To better couple the long-term effects of NF-kB activity to the cell phenotypes, we performed colony-forming assays, lasting 10 days. The extended timing of this assay allows for the evaluation of compensatory mechanisms that may develop over time, which are not apparent in the 96-hour assay. Parental and resistant cell lines were treated with CTX alone or in combination with IL-1. We observed no effect of the cytokine's stimulation alone in sensitive cells, which may suggest the exit from senescence associated growth arrest, previously detected. Nevertheless, the sensitivity to CTX inhibition was maintained also in the combination CTX and IL-1 (Fig. 4C and Supplementary Figure 4C). Resistant cells, instead, displayed a mild proliferation reduction in cells stimulated with IL-1 alone or in combination with CTX treatment (Fig. 4D and Supplementary Figure 4D). These data suggest that activation of NF-kB may assume both proliferative or antiproliferative action according to the analyzed cell type.

4. Conclusions

NF-kB signaling has been found constitutively active in many different tumors [25]. In human colorectal adenocarcinoma, patients harboring K-RAS mutations exhibit a higher NF-kB activity compared to those with wild type, which correlates with chemotherapy resistance and lower survival rates [13]. Despite the extensive use of EGFR inhibitors in the clinic and a well-established understanding of their mechanism of action, how these inhibitors modulate NF-kB signaling remain unclear. Moreover, no triggering mutations have been reported, suggesting that the aberrant NF-kB activation is likely linked to upstream signaling deregulation. Previously, our laboratory by analyzing data from 150 CRC xenopatients, revealed high IL-1 expression in the subgroup of patients resistant to CTX. Moreover, we proved that the receptor, IL-1R1, acts as a central mediator of CTX resistance in CRC, resulting highly expressed in non-responsive tumors [16]. Therefore, the identification of biological markers that may predict treatment response and understanding the molecular mechanisms underlying resistance to anti-EGFR therapy are crucial research objectives. In the current study, we assessed the NF-kB activation profile in two human colorectal adenocarcinoma cell lines, sensitive and resistant to CTX, stably expressing the luciferase reporter of NF-kB activity. We tested the effect of CTX on cell proliferation in 2D assays, confirming a significant cell viability reduction only in parental cells. Subsequently, we proceeded to examine the NF-kB activation profile, in real time. Initially, we observed a different luciferase activity: sensitive cells showed a high basal NF-kB activation, with a regular oscillatory profile, while CXR_NF-kB cells showed a basal NF-kB activation 10 log fold lower compared to sensitive cells but characterized by an irregular and high oscillation amplitude. Our analyses match the computational model computed on biochemical data derived from the average of cell population, and under these conditions, it confirms the 90 min period for NF-kB oscillation [5]. Furthermore, in sensitive cells, CTX treatment activates NF-kB signaling, amplified in the presence of IL-1. Conversely, resistant cells, growing in the presence of antibody treatment, did not show any increase of NF-kB activity suggesting a fine balanced state achieved by the cells to become drug tolerant. Only after the cytokine's stimuli, we recorded a boost of NF-kB activation. Of note, the lack of a defined peak of oscillation in resistant cells does not necessarily imply a lack of NF-kB activation, but it may be the result of a loss of negative feedback loops as well as a deficiency of cell synchronization among the analyzed population.

By narrowing down the study to the amplitude of the NF-kB period, notably the addition of IL-1 enhances the sharpness of the curve, and the amplitude of the frequency of oscillation, suggesting a synchronization in sensitive cancer cells. The CTX additive effect may be explained by its capability to drive the *de novo* transcription of IL-1 cytokines, as previously reported [21].

Interestingly, the correlation of the NF-kB profile and phenotype, suggests that the boost of NF-kB activity significantly decreases cell viability, as tested with a reduction in the number of colonies formed, particularly evident in the short proliferation assay, in sensitive cells stimulated with IL-1α and IL-1β or in combination with CTX treatment. This result may be explained with the induction of cell senescence, by IL-1 cytokines. On the contrary, resistant cells, display an increase in cell proliferation under cytokines stimuli, but the combination with CTX impairs cell growth.

To sum up, our results underscore the dependency of cancer cell phenotypes on the NF-kB activation profile, indicating that its aberrant activation may contribute to drug-resistant phenotype. Through our studies, we have demonstrated that NF-kB functions as a damped oscillator, capable of synchronizing with IL-1 stimulus derived from the external microenvironment and enhancing its amplitude in response to CTX. This property may help explain its role in promoting cancer cell survival and resistance to monoclonal antibodies.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used chatGPT in order to check and revise the grammar and readability of the text. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Donatella Romaniello: Writing – original draft, Methodology, Investigation. **Lorenzo Dall'Olio:** Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Martina Mazzeschi:** Methodology, Data curation. **Anna Francia:** Investigation, Data curation. **Federica Pagano:** Writing – review & editing, Investigation, Formal analysis. **Valerio Gelfo:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Gabriele D'Uva:** Writing – review & editing, Conceptualization. **Enrico Giampieri:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization. **Mattia Lauriola:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.slasd.2025.100219.

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