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Research Article

A Cocktail of Specific Inhibitors of HER-2, PI3K, and mTOR Radiosensitises Human Breast Cancer Cells

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Abstract

Intrinsic tumour radioresistance limits the benefit of radiotherapy. Targeted treatment modalities that are singly effective for triple-negative breast cancer are lacking, partly due to paucity of relevant targets as they are devoid of the human epidermal growth factor receptor 2 (HER-2), progesterone receptor (PR), and oestrogen receptor (ER); or to resistance to single-target therapies as a consequence of cellular heterogeneity. Concomitant targeting of cell signaling entities other than HER-2, PR and ER may sensitise triple-negative tumours to radiotherapy. In this study, we investigated the effect of an HER-2 inhibitor (TAK-165) and a dual inhibitor of phosphoinositide 3-kinase (PI3K) and mammalian target for rapamycin (mTOR) (NVP-BEZ235) in three human breast cancer cell lines. The potential of simultaneous inhibition of HER-2, PI3K and mTOR with a cocktail of the specific inhibitors TAK-165 and NVP-BEZ235, to radiosensitise human breast cancer cells *in vitro* was examined using the colony forming assay. Combined inhibition of HER-2, PI3K, and mTOR resulted in significant radiosensitisation in all cell lines, independent of HER-2, ER, or PR status. Radiosensitisation was more prominent in ER- and PR-negative cells expressing higher levels of epidermal growth factor receptor (EGFR). These data suggest that a cocktail of TAK-165 and NVP-BEZ235 could potentially be effective in the treatment of triple-negative breast cancer.

Keywords: Triple-negative, breast cancer, targeted therapy, radiosensitisation.

Introduction

Many subtypes of breast cancer exist and exhibit

unique characteristics. Different, as well as, specific treatment modalities would be required to benefit all subgroups. Cancers overexpressing the gene encoding the human epidermal growth factor receptor 2 (HER-2) constitute 30% of invasive breast cancers [1]. Approaches for targeting HER-2 are important in the treatment of breast cancers overexpressing HER-2. Although trastuzumab has been approved by the United States Food and Drug Administration (US FDA) for the treatment of HER-2 positive cancers, a significant level

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of resistance to trastuzumab treatment is apparent [2]. Dysregulation of downstream components of the HER-2 signaling pathway have been suggested to be responsible for the observed resistance [2]. Such resistance may also be partly attributable to the heterogeneity in the distribution of target antigen expression in a given cell population, which can lead to the inability to effectively target all cells with toxic levels of therapeutic agents [3]. Another challenge is that some HER-2 positive cancers express a constitutively active truncated form of the protein (p95 HER-2) which does not possess the extracellular domain required for trastuzumab binding [4], and HER-2 targeted treatment may fail.

Also, about one-fifth of women with breast cancer constitutes the subgroup of patients with triple-negative breast cancer [5]. This subtype of breast cancer is known to occur more frequently in young Black and Hispanic women than in young women of other racial or ethnic groups [6]. Triple-negative breast cancers lack expression of HER-2, progesterone receptor (PR), and oestrogen receptor (ER), all of which are promising candidates for targeted therapy. Therefore, patients with triple-negative tumours cannot be treated with endocrine based therapy or therapies targeting HER-2, and treatment outcome in these individuals is relatively poor [7]. To improve the management of triple-negative breast cancer, effective targeting of malignant cells devoid of ER, PR, and HER-2 expression is warranted.

An alternative treatment modality for breast cancer is radiotherapy. Cellular exposure to ionising radiation is known to activate the epidermal growth factor receptor, EGFR [8], and induce phosphoinositide 3-kinase (PI3K), serine-threonine protein kinase (Akt) and mammalian target for rapamycin (mTOR) activity downstream of the EGFR signaling pathway [9]. This signaling promotes cell survival and can lead to treatment resistance. Targeting the HER-2 pathway by inhibiting PI3K has also been shown to result in significant radiosensitisation [10]. Of significance to targeted therapy, triple-negative breast cancers, which are devoid of HER-2 activity, tend to predominantly overexpress EGFR [11]. Therefore, developing therapeutic approaches that concomitantly target EGFR family members and their downstream signaling components might significantly sensitise

triple-negative breast cancer cells to radiotherapy and improve treatment outcome.

In the present work, studies were conducted to determine if inhibition of HER-2, PI3K and mTOR can radiosensitise human breast cancer cells. To achieve this, MDA-MB-231, MCF-7 and MCF-12A cells were treated with TAK-165 (an HER-2 inhibitor) and NVP-BEZ235 (a dual inhibitor of PI3K and mTOR) and concomitantly irradiated, and radiation-induced cell death was measured using the colony forming assay. The dependence of radiosensitisation on HER-2, ER and PR expression status and the implications of inhibiting the HER-2/PI3K/mTOR pathway in irradiated breast cancer cell lines are also discussed.

Materials and Methods

Cell lines and culture maintenance

The MDA-MB-231 and MCF-7 human breast tumour cells were derived from pleural effusions taken from women with invasive ductal adenocarcinoma and carcinoma, respectively, and were a gift from Prof S Prince (University of Cape Town, South Africa). They respectively form metastatic xenografts in nude mice in an oestrogen-independent and dependent manner. The immortalised mammary epithelial cell line, MCF-12A, was a gift from Prof AM Engelbrecht (University of Stellenbosch, South Africa). The rationale for choosing these cell lines is that they significantly differ in expression of potential target antigens, such as, HER-2, ER, PR and EGFR. Although MCF-7 cells are EGFR, HER-2, ER and PR positive [12,13], their expression of HER-2 is low and comparable to that in the MDA-MB-231 cells [14] and MCF-12A cells [15]. While expression of EGFR in MCF-7 cells is also low, MDA-MB-231 and MCF-12A cells which are known to be ER and PR negative [15], express higher levels of EGFR [14,15]. Furthermore, the MDA-MB-231 and MCF-12A cell lines express wild-type PI3K, whereas the MCF-7 cell line is PI3K mutant [16,17]. The MDA-MB-231 and MCF-7 cell lines were routinely cultured in Roswell Park Memorial Institute (RPMI-1640) medium (Sigma-Aldrich, USA). MCF-12A cells were cultured in Dulbecco's Modified Eagle medium (DMEM) nutrient mixture F-12 Ham (Sigma-Aldrich, USA), supplemented

with 20 ng/ml human epidermal growth factor (Sigma-Aldrich, Germany, cat # E9644), 0.01 mg/ml bovine insulin (Sigma-Aldrich, Germany, cat # I5500), and 500 ng/ml hydrocortisone (Sigma-Aldrich, Germany, cat # H0888). All growth media were further supplemented with 10% heat-inactivated foetal bovine serum (FBS) (HyClone, UK), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Lonza, Belgium). Cell cultures were routinely incubated at 37°C in a humidified atmosphere (95% air and 5% CO₂). Cells were grown as monolayers in 75-cm² flasks (Greiner Bio-One, Germany, cat # 658170) and were used for experiments (passages 18-39) upon reaching 80-90% confluence.

Target inhibitors

TAK-165 (Tocris Bioscience, UK, cat # 3599) is a specific inhibitor of HER-2. NVP-BEZ235 is a dual inhibitor of PI3K and mTOR (Santa Cruz Biotechnology, TX, USA, cat # 364429). Stock solutions of TAK-165 (21 mM) and NVP-BEZ235 (106 mM) were prepared in dimethyl sulfoxide and stored at 4°C and -20°C, respectively, until used.

Cell survival assay and radiosensitivity

The colony assay was used to measure intrinsic radiation response in all cell lines. Cultures in exponential growth were trypsinised to give single-cell suspensions and were plated (500-10000 cells per flask, adjusted for irradiation dose) into 25 cm² culture flasks (Greiner Bio-One, Germany, cat # 690160), and incubated for 3-4 h to allow the cells to attach. Cell cultures were then irradiated to 0-10 Gy with ⁶⁰Co γ-rays and reincubated. The mean dose rate used in this investigation was 0.83 Gy/min (range: 0.78-0.87 Gy/min). Cultures were irradiated at room temperature (22°C). After growing for 7-10 days, depending on the cell line, colonies were fixed in glacial acetic acid:methanol:water (1:1:8, v/v/v), stained with 0.01% amido black in fixative, washed in tap water, air-dried, and were counted. Three independent experiments were performed for each dose point, and the mean surviving fractions were fitted to the linear-quadratic (LQ) model to generate survival curves. Cellular radiosensitivity was expressed in terms of the surviving fraction at 2 Gy (SF₂).

Target inhibitor toxicity measurements

Single-cell suspensions were plated (1000-4000 cells per flask) into 25 cm² culture flasks, and incubated for 3-4 h to allow the cells to attach. To assess the influence of inhibitor concentration on cytotoxicity, cells were exposed to TAK-165 (3.7-137.4 nM) and NVP-BEZ235 (0.6-69.2 nM) and incubated for 7-10 days for colony formation. The colonies were fixed in glacial acetic acid:methanol:water (1:1:8, by volume), stained with 0.01% amido black in fixative, washed in tap water, air-dried, and counted. To determine the equivalent concentration of each inhibitor for 50% cell kill (EC₅₀), the surviving fractions (SF) were plotted as a function of log(inhibitor concentration) and were fitted to a 4-parameter logistic equation of the form:

$$SF = B + \frac{T-B}{1-10^{[(\log EC_{50}-X)HS]}} \quad (1),$$

where *B* and *T* are the minimum and maximum of the sigmoidal curve, respectively, *X* is the log(inhibitor concentration), and *HS* is the steepest slope of the curve. Three independent experiments were performed for each cell line and dose point.

Determination of radiosensitivity modification by TAK-165 and NVP-BEZ235

To investigate the influence of inhibitor exposure on radiosensitivity, attached cells were treated with 30 nM of TAK-165 (~EC₅₀ predetermined for MDA-MB-231 and MCF-7 cell lines) and 17 nM of NVP-BEZ235 (~4 × EC₅₀ for MDA-MB-231 and MCF-7 cell lines), or a cocktail of both inhibitors at the same concentrations, and irradiated immediately with 2 Gy, the typical dose per fraction in conventional radiotherapy, using ⁶⁰Co γ-rays. The use of a relatively high NVP-BEZ235 concentration was to ensure adequate inhibition of the dual targets, as these would be expected to present a larger number of binding sites. For each experiment, sets of cell culture flasks given inhibitors alone (singly and in combination) and unirradiated flasks without inhibitors served as controls for cultures irradiated with and without inhibitors, respectively. Inhibitor-treated cell cultures were used as controls for those receiving inhibitors and irradiation to allow for interexperimental variations

in inhibitor toxicity, as exposures to predetermined concentrations do not always yield the expected cell kill. The interaction between inhibitors and γ -irradiation (2 Gy) was expressed as a modifying factor (MF), which is given by the ratio of surviving fractions (SF) in the absence and presence of inhibitors as follows:

$$MF = \frac{SF(2\text{ Gy})}{SF(\text{inhibitor} + 2\text{ Gy})} \quad (2).$$

The criteria for inhibition, no effect, and enhancement of radiosensitivity by inhibitors are $MF < 1.0$, $MF = 1.0$ and $MF > 1.0$, respectively.

Data analysis

Statistical analyses were performed using the GraphPad Prism (GraphPad Software, San Diego, CA, USA.) computer program. Standard equations were used to fit nonlinear relationships. Data were calculated as the means ($\pm$ SE) from three independent experiments. For each experiment and data point, 3 replicates were assessed. To compare two data sets, the unpaired *t*-test was used. *P*-values and coefficients of determination, R^2 , were calculated from two-sided tests. A *P*-value of <0.05 indicates a statistically significant difference between the data sets.

Results

Intrinsic radiosensitivity of MDA-MB-231, MCF-7, and MCF-12A cells

Cellular radiosensitivity expressed in terms of the SF_2 was determined from clonogenic cell survival data (Figure 1). SF_2 -values were obtained by fitting the mean surviving data to the linear-quadratic model. From the cell survival data in Table 1, the intrinsic SF_2 -values for the MDA-MB-231 (PI3K wild-type), MCF-7 (PI3K mutant) and MCF-12A (PI3K wild-type) emerged as 0.59 ± 0.07 , 0.23 ± 0.01 , and 0.60 ± 0.07 , respectively. The MDA-MB-231 and MCF-12A cell lines show similar radiation response at 2 Gy and are deemed radioresistant, whereas the MCF-7 cell line is deemed radiosensitive.

Cytotoxicity of TAK-165 and NVP-BEZ235

Treatment of cells with inhibitors alone induced a concentration-dependent cell kill (Figure 2). At cell survival rates ranging from 20-90%, NVP-BEZ235 was clearly more potent than TAK-165 in both MDA-MB-231 and MCF-7 cell lines. The equivalent concentrations of TAK-165 and NVP-BEZ235 for 50% cell survival for the MDA-MB-231 cell line were found to be 4.25 ± 0.23 nM (95% CI: 3.79-4.76 nM) and 27.12 ± 4.65 nM (95% CI: 18.50-39.74 nM), respectively. The corresponding EC_{50} -values for the MCF-7 cell line emerged as 4.15 ± 0.34 nM (95% CI: 3.48-4.95 nM) and 24.88 ± 2.26 nM (95% CI: 20.32-30.45 nM), respectively.

Modulation of radiosensitivity by TAK-165 and NVP-BEZ235

To evaluate the impact of inhibitor exposure on radiosensitivity, based on clonogenic cell survival, cell cultures were irradiated to 2 Gy immediately after administering TAK-165, NVP-BEZ235, or a combination of both inhibitors (Figure 3). In MDA-MB-231 cells (Figure 3A), inhibition of HER-2 with TAK-165 alone led to a small and insignificant radiosensitisation, with SF_2 decreasing from 0.59 ± 0.01 to 0.49 ± 0.05 ($P=0.13$, $R^2=0.48$). This translated to about 20% increase in radiotoxicity (Table 1). In contrast, treatment of MCF-7 and MCF-12A cells did not appear to affect cellular radiosensitivity at 2 Gy (Figures 3B and 3C and Table 1). Inhibiting PI3K and mTOR activity with NVP-BEZ235 significantly radiosensitised MDA-MB-231 and MCF-7 cells, but not the apparently normal MCF-12A cells (Figure 3 and Table 1). The resulting SF_2 -values were 0.32 ± 0.04 ($P=0.0046$, $R^2=0.89$), 0.14 ± 0.02 ($P=0.02$, $R^2=0.78$) and 0.46 ± 0.05 ($P=0.25$, $R^2=0.57$) for the MDA-MB-231, MCF-7 and MCF-12A cell lines, respectively. The corresponding modifying factors were 1.84 ± 0.23 , 1.64 ± 0.05 and 1.30 ± 0.21 . Concomitant treatment with TAK-165 and NVP-BEZ235, significantly enhanced radiosensitivity in all cell lines ($0.0002 \leq P \leq 0.0183$), yielding ~4-, ~3-, and ~7-fold reduction in cell survival in the MDA-MB-231, MCF-7 and MCF-12A cell lines, respectively (Table 1).

Figure 1: Clonogenic cell survival curves for 3 human breast cell lines after ^{60}Co γ -irradiation. Symbols represent the mean surviving fraction $\pm$ SE from three independent experiments. Standard errors are not transformed into a logarithmic scale. Survival curves were obtained by fitting experimental data to the LQ model.

Figure 2: HER-2, PI3K and mTOR inhibitor toxicity in 3 human breast cell lines expressed as cell survival and plotted as a function of $\log(\text{inhibitor concentration})$. Cell survival was determined by the colony assay, and data were fitted to a 4-parameter logistic equation. Data points are means $\pm$ SE of 3 independent experiments.

Discussion

The phosphatase and tensin homolog (PTEN) which impedes PI3K/mTOR pathway activity, thereby sensitising cells to cytotoxic insult, is widely expressed in all cell lines used here [16]. Therefore, the observed differences in radiosensitivity, as illustrated in Figure 1, cannot be attributed to disparities in PTEN activity. While the more radioresistant MDA-MB-231 and MCF-12A cell lines express wild-type PI3K, the radiosensitive MCF-7 cells are PI3K mutant [16,17]. This may explain the relatively high radiosensitivity in the latter cell line. A reduction in PI3K/mTOR signaling in MCF-7 cells due to the PI3K mutation can compromise their ability to recover from radiation-induced damage.

It is demonstrated for MDA-MB-231 and MCF-7 cells that cytotoxicity of NVP-BEZ235 and TAK-165, based on clonogenic survival, is concentration-dependent (Figure 2). For NVP-BEZ235 treatment, the EC_{50} -values of 4.25 and 4.15 nM obtained for MDA-MB-231 and MCF-7, respectively, are comparable with PI3K/mTOR inhibition data reported elsewhere for MDA-MB-231 and the HER-2 amplified breast cancer cell lines BT474 and MDA-MB-175-VII [16,17]. In contrast, significantly higher NVP-BEZ235 concentrations for 50% growth inhibition (IC_{50}) ranging from 6-93 nM have emerged for many other breast cancer cell lines, with HER-2 amplified cell lines tending to be more sensitive [16,18]. Interestingly, Brachmann et al. [18] demonstrated that doses of NVP-BEZ235 for 50% cell kill (LD_{50}) for MDA-MB-231 and MCF-7 cells can be ~87 and >20 000 nM, respectively. The disparity in toxicity noted here can be explained by the fact that cell growth and metabolic assays which extend over relatively short periods often tend to overestimate cell survival following cytotoxic treatment. Cell growth and metabolic assays are snapshots of cellular demise and may not adequately reflect residual cellular reproductive integrity as measured by the colony forming assay; and the resulting LD_{50} -values can be unrealistically high. The similarity in NVP-BEZ235 toxicity in MDA-MB-231 and MCF-7 cells seems to suggest that NVP-BEZ235-induced cell death cannot be attributed to ER-mediation of PI3K activity, as the latter cell line is known to exhibit ER-dependent PI3K activity while the former does not [19]. Based on clonogenic cell survival, TAK-165 was generally

less potent, with an EC_{50} of ~25-27 nM (Figure 2). All cell lines minimally express HER-2 [13,14], and the extensive concentration-dependent TAK-165 induced cytotoxicity observed here cannot be solely attributed to HER-2 alone. This finding is likely due to targeting of residual HER-2, as well as, other critical cellular factors. TAK-165 is a potent inhibitor of EGFR and the cell division cycle protein 2 homolog (Cdc2), which play a crucial role in cell-cycle progression. Perturbation of their activity with TAK-165 can lead to cellular demise during cell division. Cdc2 activity in MDA-MB-231 is intrinsically higher than that in MCF-7 [20], indicating a stronger dependence of the former cell line on Cdc2 activity for cell cycle progression. Inhibiting Cdc2 and the residual HER-2 activity with TAK-165 can, therefore, be expected to be more toxic in MDA-MB-231 cells than in their MCF-7 counterparts. However, TAK-165 concentrations used in the current study are much lower than those that are typically required to significantly suppress Cdc2 and EGFR activity [21]. At such high concentrations, TAK-165 has been shown to be ~4-fold more inhibitory than demonstrated here in a variety of cancer cell lines of bladder, kidney and prostate origin in which HER-2 expression ranged from weak to high [21].

In this presentation, it is demonstrated that treatment of all the three cell lines with TAK-165 yielded only 6-20% radiosensitisation (Figure 3 and Table 1). This is not unexpected, as the cell lines express very low levels of HER-2 [14,15]. The role of HER-2 perturbation in radiosensitivity modulation should, therefore, be minimal. This is consistent with data reported elsewhere indicating that trastuzumab (a potent HER-2 inhibitor) had little or no effect on radiation-induced apoptosis in breast cancer cell lines that show low to no expression of HER-2 [22]. However, when cells were treated with the PI3K/mTOR inhibitor, significant radiosensitisation was seen in the MDA-MB-231 and MCF-7 cell lines, but not in the MCF-12A cell line (Figure 3 and Table 1). These data cannot be explained in terms of PI3K status, as NVP-BEZ235 induced radiosensitisation was seen in the MDA-MB-231 (PI3K wild-type) and MCF-7 (PI3K mutant) cell lines, but not in the MCF-12A (PI3K wild-type) cell line. The marked disparity between ~2-fold radiosensitisation observed here and the ~4-fold radiosensitisation reported elsewhere [23],

Figure 3: Clonogenic cell survival at 2 Gy (SF_2) for 3 human breast cell lines after ^{60}Co γ -irradiation: (A) MDA-MB-231, (B) MCF-7, and (C) MCF-12A. Cells were irradiated without or in the presence of TAK-165 (HER-2 inhibitor) and NVP-BEZ235 (dual inhibitor of PI3K and mTOR), either administered singly or in combination. Bars represent the mean surviving fraction $\pm$ SE from three independent experiments. In comparison with SF_2 without inhibitors: * $0.005 < P \leq 0.02$; ** $P \leq 0.005$.

might be due to differences in experiment design. The NVP-BEZ235 concentration of 100 nM used by Kuger and colleagues was ~ 6 times that used in the present study [23], and corresponds to NVP-BEZ235 doses at which cell survival levels should become very low if the residence time of the drug was over the entire colony forming period (Figure 2). Drug cytotoxicity strongly depends on residence time [24]. Also, delayed trypsinisation and re-plating of cells after drug and radiation treatment can significantly modify the extent

to which the drugs modulate radiosensitivity.

Interestingly, although radiosensitisation by TAK-165 was expectedly minimal, a 3- to 6-fold radiosensitisation emerged when cell cultures were pre-treated with a combination of TAK-165 and NVP-BEZ235 (Table 1). To interrogate any potential mode of interaction between the two inhibitors, the data presented in Figure 2 were used to construct median-effect plots, as illustrated in Figure 4, from which combination indices (CI) were

Figure 4: Median-effect plots for 2 human breast cell lines, treated with NVP-BE235 (circles and solid lines) and TAK-165 (squares and dashed lines), from toxicity data presented in Figure 2: (A) MDA-MB-231 and (B) MCF-7. Transformed data were fitted to the function: $\log(f_u/f_u) = m \times \log(D) - m \times \log(D_m)$, where f_a and f_u are the affected and unaffected fractions of cells, respectively, and the coefficient m is an indicator of the shape of the inhibitor concentration-effect relationship ($m=1$, >1 , and <1 indicate hyperbolic, sigmoidal, and flat-sigmoidal inhibitor concentration-effect curves, respectively), D_m is the median-effect concentration of inhibitor, and D is the concentration of inhibitor [25]. Horizontal dotted lines are the median-effect axes. For MDA-MB-231, the fitted m and D_m values were 1.78 and 7.28 nM, respectively, for NVP-BE235 ($r=0.93$); and 2.16 and 24.72 nM, respectively, for TAK-165 ($r=0.95$). The corresponding values for MCF-7 were 2.05 and 5.22 nM for NVP-BE235 ($r=0.98$); and 2.18 and 20.51 nM for TAK-165 ($r=0.95$). The r -values were high, signifying a strong conformity of the data to the mass-action law [25]. D_m values were marginally lower than corresponding EC_{50} -values.

estimated for the single inhibitor cocktail for the MDA-MB-231 and MCF-7 cell lines [25]. CI-values of 0.72 and 0.73 emerged for the MDA-MB-231 and MCF-7 cell lines, respectively, indicating synergism for TAK-165 and NVP-BE235 at the concentrations used here. This might explain the more than additive enhancement of radiosensitivity seen in all cell lines (Table 1). Of specific note is the ~7-fold radiosensitisation observed in the MCF-12A cell line when cells were pre-treated with inhibitor cocktail. It is not likely that the inhibitory activity of the TAK-165/NVP-BE235 cocktail is directed towards ER and PR, as radiosensitisation was higher in the ER-negative and PR-negative cell lines (MDA-MB-231 and MCF-12A) than the ER and PR overexpressing MCF-7 cell line [14,15]. The current findings seem to suggest that the activity of the TAK-165/NVP-BE235 cocktail is specific for other targets, such as, EGFR which is often overexpressed by cells with compromised activity of HER-2, ER and PR [11]. The rank order of EGFR expression in the three cell lines is: MCF-7<MDA-MB-231<MCF-12A [14,15], and is consistent with the extent of radiation dose

modification (Table 1). Therefore, EGFR is a likely target for a TAK-165/NVP-BE235 cocktail. The higher radiosensitisation seen in the intrinsically radioresistant immortalised mammary epithelial cell line (MCF-12A), following the cocktail treatment, might be an indication that concomitant use of cocktail and radiation could potentially elevate normal tissue toxicity and requires further evaluation.

In conclusion, this study demonstrates that concomitant inhibition of HER-2, PI3K, and mTOR in human breast cancer cell lines with differing HER-2, ER, PR, and EGFR expression levels results in significant radiosensitisation on the basis of clonogenic cell survival. Radiosensitisation is more prominent in ER- and PR-negative cells expressing higher levels of EGFR. These findings suggest that a cocktail of TAK-165 and NVP-BE235 may have the potential for more effectively targeting triple-negative breast cancer cells, and provide the basis for further studies involving a larger panel of cell lines covering a wider range of HER-2, ER, PR, and EGFR expression profiles.

Table 1: Summary of radiosensitivity and dose modifying data for three human breast cell lines treated with inhibitors TAK-165 (for HER-2) and NVP-BEZ235 (for PI3K and mTOR)

Cell line	Treatment	SF_2^*	$MF_2^{\#}$
MDA-MB-231	2 Gy	0.59 ± 0.01	
	2 Gy + TAK-165	0.49 ± 0.05	1.20 ± 0.13
	2 Gy + NVP-BEZ235	0.32 ± 0.04	1.84 ± 0.23
	2 Gy + NVP-BEZ235 + TAK-165	0.15 ± 0.03	3.93 ± 0.79
MCF-7	2 Gy	0.23 ± 0.01	
	2 Gy + TAK-165	0.22 ± 0.02	1.06 ± 0.11
	2 Gy + NVP-BEZ235	0.14 ± 0.02	1.64 ± 0.05
	2 Gy + NVP-BEZ235 + TAK-165	0.08 ± 0.04	2.86 ± 1.44
MCF-12A	2 Gy	0.60 ± 0.07	
	2 Gy + TAK-165	0.55 ± 0.01	1.09 ± 0.13
	2 Gy + NVP-BEZ235	0.46 ± 0.05	1.30 ± 0.21
	2 Gy + NVP-BEZ235 + TAK-165	0.09 ± 0.01	6.67 ± 1.07

SF_2 and MF_2 denote the surviving fraction and radiation modifying factor at 2 Gy, respectively. *Mean $\pm$ SEM.

$^{\#}$ Mean $\pm$ error: errors were calculated using appropriate error propagation formulae.

Author Disclosures

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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