p53 inactivation decreases dependence on estrogen/ERK signalling for proliferation but promotes EMT and susceptibility to 3-bromopyruvate in ERα+ breast cancer MCF-7 cells

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Background: Most breast cancers express the estrogen receptor alpha (ERα+), harbor wt TP53, depend on estrogen/ERK signalling for proliferation, and respond to anti-estrogens. However, concomitant activation of the epidermal growth factor receptor (EGFR)/MEK pathway promotes resistance by decreasing estrogen dependence. Previously, we showed that retroviral transduction of mutant p53 R175H into wt TP53 ERα+ MCF-7 cells induces epidermal growth factor (EGF)-independent proliferation, activation of the EGF receptor (p-EGFR) and some characteristics of epithelial-mesenchymal transition (EMT).

Purpose: To investigate whether p53 inactivation augments ERα+ cell proliferation in response to restrictive estradiol, chemical MEK inhibition or metabolic inhibitors.

Results: Introduction of mutant p53 R175H lowered expression of p53-dependent PUMA and p21WAF1, decreased E-cadherin and cytokeratin 18 associated with EMT, but increased the % of proliferating ERα+/Ki67 cells, diminishing estrogen dependence. These cells also exhibited higher proliferation in the presence of MEK-inhibitor UO126, reciprocally correlating with preferential susceptibility to the pyruvate analog 3-bromopyruvate (3-BrPA) without a comparable response to 2-deoxyglucose. p53 siRNA silencing by electroporation in wt TP53 MCF-7 cells also decreased estrogen dependence and response to MEK inhibition, while also conferring susceptibility to 3-BrPA.

Conclusions: (a) ERα+ breast cancer cells dysfunctional for TP53 which proliferate irrespective of low estrogen and chemical MEK inhibition are likely to increase metabolic consumption becoming increasingly susceptible to 3-BrPA; (b) targeting the pyruvate pathway may improve response to endocrine therapy in ERα+ breast cancer with p53 dysfunction.

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1. Introduction

Breast cancer is an important cause of death in women with prevalence increasing with age [1]. Hormone receptor status is one of the main differentiating characteristics of human breast cancers and modifies therapeutic response. Estrogen receptor alpha (ERα) signaling plays an important role in many tissues, including the mammary gland, where it is required for normal gland development and maintenance. About 65% of human breast cancers are ERα positive [1–3]. When dysregulated, ERα induces abnormal proliferation helping in the initiation and progression of breast cancer [2,3]. Frequent co-expression of ERα+ and the proliferation-associated marker Ki-67 occurs with ERα+ breast cancer progression, but this is rare in the normal premenopausal human breast [2,3]. Genetic changes affecting the tumor suppressor p53 gene are reported in about 25% of human breast cancers [4,5]. Mutant p53 R175H is a hotspot mutation in the zinc-binding region, which loses p53-dependent tumor-suppressor activities, but is believed to acquire new oncogenic promoting functions [6,7]. Despite the correlation of p53 cancer mutations and poor prognosis of human breast cancer patients [6–8], the roles of p53 cancer mutants in promoting breast cancer and their relationship to estrogens are

Abbreviations: mutant p53 R175H, mutant p53 Arg 175His; wt, wild type; ERα, estrogen receptor alpha; 3-BrPA, 3-bromopyruvate; E2, estradiol; EMT, epithelial-mesenchymal transition.  
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still being elucidated [8–11]. ERα binds the p53 tumor suppressor protein directly and represses its function [8]. However, despite harboring wild-type p53, ERα+ breast cancer cells are resistant to chemotherapy-induced apoptosis in the presence of estrogen [9–14]. Epithelial–mesenchymal transdifferentiation (EMT) involving loss of E-cadherin with disruption of epithelial cytoarchitecture from a cobblestone-like appearance to an elongated mesenchymal phenotype, is another characteristic of breast cancer progression involving mutant p53R175H [15].

Previously, we showed that loss of E-cadherin characteristic of EMT, EGF-independent proliferation and activation of EGFR were augmented in mutant p53 transduced MCF-7 cells compared to their genetically-matched wt p53 counterparts [15]. A single copy of point-mutated p53 suffices to make mammary cells sensitive to the EMT response driven by TGF β [16] and matrix-metalloproteinase activation [17–19]. EMT as an important morphologic and developmental change involves an extensive cytoskeletal rearrangement [20,21] including de novo expression of mesenchymal vimentin [22].

Cytoskeletal reorganization in EMT and breast cancer progression also can include down-regulated expression of cytokeratin 18 [20,23]. Restoration of cytokeratin 18 in human breast cancer cells induces a dramatic regression of malignancy in vitro and in vivo [20,22,24] possibly because keratin 18 expression is also involved in EMT-driven associated signaling [25]. A study using estrogen-dependent breast cancer cell lines with different p53 status revealed that p53 mutated cells were more resistant to cytotoxic effects of 4-hydroxy-tamoxifen (OHT) compared to p53 wild-type cells [4].

Estrogenic activation is likely to induce MEK/ERK signaling and downstream phosphop90RSK expression, reflecting the importance of the Ras/Raf/ERK/ p90RSK pathway activation signal in the response of ER-positive breast cancer [26–30].

Blockade of the MAPK/ERK signaling cascade with the MEK inhibitor U0126 inhibits ERα activity via enhanced recruitment of the co-repressor SMRT, leading to reduced expression of ERα target genes and to slower growth rate of MCF-7 cells treated with both TAM and U0126 [29,30]. Since p53 mutations [6,7], aberrant ERα/MEK/ERK signaling [8–13,26–32] and resistance to genotoxic stress [33] cooperate to promote breast cancer, in this paper we investigated in genetically-matched ERα+ MCF-7 cells, whether mutant p53 R175H increases EMT, secretion of matrix metalloproteinases [34] and loss of cytokeratin 18 [20,23–25] together with augmenting proliferation independently of estradiol and MEK signalling. Since ERα+ breast cancer cells develop resistance to endocrine therapies by a mechanism involving estrogen receptor (ERα)-regulated and growth factor receptor-regulated survival pathways [35] and Bcl-2 over-expression in human breast cancer cells [36], we also investigated whether specific Bcl-2/Bcl-xl antisense sequences [37] influence ERα-linked proliferation in a p53-dependent manner. Finally, since glucose consumption may be stimulated by hyper-proliferation under restrictive amounts of exogenous estrogen and EGF [15,36,38,39], we have now investigated whether ERα+ breast cancer cells differing in p53 status respond unequally to metabolic antagonists like the glycolytic inhibitor 2-deoxyglucose [40] or to 3-bromopyruvate (3-BPa), an agent that potentially may inhibit both glycolysis and mitochondrial function [41–43].

2. Materials and methods

2.1. Retroviral transduction and verification of p53 status in cells

Experiments were performed with MCF-7 human breast carcinoma cells obtained from the ATCC. These cells harbour a functional wt p53, as evidenced by high activation of the p21WAF1 gene product [15,33,44]. To obtain p53 mutant cells, parental MCF-7 cells were retrovirally transduced as follows: Phoenix cells were transfected with the pWZL-Hygro plasmid harbouring a human p53 histidine-175 mutant gene, a dominant-negative p53 mutant plasmid, in the presence of calcium chloride, as previously described [15]; control cells were retrovirally transduced with the empty pWZL-Hygro plasmid.

2.2. Transient p53 siRNA silencing

This was carried out using the Neon electroporation system (Invitrogen Cat No. MPK5000, Carlsbad, CA, 92208, USA) containing 1.5 × 105 cells resuspended in 100 μl buffer R (Cat No. MPK10025R) and 4 μl containing 10 nM of p53 siRNA (H) duplex standardized to silence p53 (Cat # SC-29435). Cells were treated in parallel with a comparable concentration of negative control siRNA-A (Cat # SC-36868), a non-targeting duplex of the same length as the specific p53 siRNA. Both siRNA types were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, 95060, USA). Cells were subjected to electroporation using 2 pulses (width 30 and 1150 voltage) and subsequently seeded to 35% confluence in medium devoid of antibiotics in 96 well plates. After 12 h, cultures were washed and subjected to the indicated treatments in charcoal-treated estrogen-depleted medium for further assays within 48 h.

2.3. Relative cell viability/metabolic activity

This was estimated with Alamar Blue (Catalog Number DAL1025, Life Technologies, Carlsbad, CA 92208, USA), which measures intracellular redox mitochondrial activity by quantitating the cell-catalyzed conversion of non-fluorescent resazurin to fluorescent resorufin [33]. Alamar Blue was added to a 10% final concentration to each one of 96 well plates after the appropriate treatment, the dye is non-toxic, allows fluorescent quantitation, permits re-use for further investigation such as morphological, biochemical and clonogenic analyses. As such, this assay is valuable as an endpoint of proliferation or relative viability/metabolic activity, rather than a kinetic measure for monitoring cell growth. For these experiments, cells (10,000) were allowed to adhere overnight in 96 well TC microtiter dishes. After the corresponding treatments, Alamar Blue was added without removing medium containing dead cells, and fluorescence was measured 4 h later in a Fluoroskan Ascent microplate reader with an excitation of 544 nm and an emission of 590 nm.

2.4. Immune blotting

Cells were harvested in PBS containing protease and phosphatase inhibitors using a rubber policeman and lysates were obtained, as described [33]. Extracts were prepared in cell lysis buffer (50 mM Tris–HCl, pH 8, 120 mM NaCl, 50 mM NaF, 0.1 mM sodium vanadate, 5 mM EDTA, 10 μg/mL each of leupeptin, soybean trypsin inhibitor, and aprotinin, 1 mM phenylmethylsulfonyl fluoride, 0.4% Nonidet P-40). 50 μg of protein per lane run in parallel with prestained MW markers (Life Technologies, Gaithersburg, Md 20877, USA) was loaded into each well of an 11% SDS–polyacrylamide gel and electrophoretically separated. Proteins in the gels were transferred bi-directionally by diffusion blotting onto replicate nitrocellulose membranes [33]. After protein transfer, the membranes were blocked for 2 h at room temperature with TBS (Tris-buffered saline, pH 7.5) containing 0.1% Tween–20 and 5% nonfat skim milk, and membranes were reacted overnight with specific antibodies in the same blocking solution. Whenever necessary, blot pairs were erased by heating (70°C; 30 min) with 62 mM Tris–HCl, 2% SDS, 100 mM β-mercaptoethanol. The stripped blots were then washed extensively with 0.1%
TWEEN-20 in TBS, blocked with skim milk, and exposed to a different set of primary antibodies. In each case, detection was accomplished by reaction with peroxidase-conjugated secondary antibody and Super Signal chemiluminescence (Pierce Chemical, Rockford, IL 61105, USA). Antibodies used for specific immune blotting were rabbit antibodies to p21WAF1 (SC-397), PUMA (SC-28226), mouse monoclonal antibodies to p53 (DO-1; SC-126), cyclin A (SC-751), Ki67 (SC-15-402), and E-cadherin (SC-8426), all obtained from Santa Cruz Biotechnology (Santa Cruz, CA 95060, USA). Monoclonal antibody to actin (Cat # CP01, JLA-20) was from Oncogene Research (San Diego, CA 92121, USA). After extensive washing to remove nonspecific binding, membranes were reacted with anti mouse IgG-peroxidase for detection of mouse antibodies, or with Protein A-peroxidase for detection of rabbit antibodies. Loading control bands came from the stripped blot of the target protein, or from replicate nitrocellulose from the bi-directional blots. Densitometric quantitation of differential protein expression was achieved by digitalization of images, avoiding saturation density and choosing adequate dynamic range with a Fluor-S Imager (Bio-Rad, Hercules, CA 94547, USA). This was followed by quantitation of specific bands with the Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD 20850, USA).

2.5. Crystal violet staining of surviving cells

Cells were subjected to 3-bromopyruvate, at the concentrations indicated in each experiment. Subsequently, the unattached dead population was removed after washing twice in isotonic phosphate-buffered saline. Surviving cells were evidenced following fixation in 90% ethanol and cell staining with 0.5% crystal violet (Cat # C-3886, Sigma–Aldrich, St. Louis, MO 63103, USA) in 30% ethanol.

2.6. Laser scanning cytometry (LSC)

An LSC-2 cytometer (Compucyte, Cambridge, MA 02139, USA), which measures fluorescence of individual cells contoured on the basis of nuclear DNA counterstain with propidium iodide was used. Every sample was scanned using identical non-saturating fluorescence settings, to allow quantitative comparisons to be made [15]. To analyze fluorescence changes only on individual cells, clustered aggregates were gated out, to quantitate integral (total fluorescence within the integral contour) and maximal pixels (highest localized fluorescence within the threshold contour). After the indicated culture condition, cells attached to LabTek multiwell plates were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), followed by permeabilization with 0.25% Triton X-100, washing in PBS, blocking in 5% albumin (in PBS), then reacted with monoclonal antibodies to human cyclin A (SC-751), Ki67 (SC-15-402), and ERα (SC-542) from Santa Cruz Biotechnology (Santa Cruz, CA 95060, USA). All these antibodies were validated for specific immune fluorescence. Negative controls were stained with secondary antibody without primary antibody. Multi-label detection was achieved by reaction with corresponding secondary antibodies conjugated to either Oregon Green (Molecular Probes, Eugene, OR, USA) for excitation with an Argon laser or Cy5 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) for Long Red detection with an HeNe laser. Integral contouring was done by staining DNA with propidium iodide in the red region [15], using the corresponding blocking filters.

2.7. Immunofluorescence

Subconfluent cultures were seeded in complete medium on 8-well LabTek plates pre-coated with 5 μg/ml fibronectin. When

Fig. 1. Mutant p53 R175H induces epithelial–mesenchymal transition, extracellular MMP-2 and loss of cytokeratin 18. (A) Vector-transduced MCF-7 cells or those transduced with mutant p53 R175H were seeded in Lab-Tek 8 well slides and allowed to grow for 3 days, following by fixation and permeabilization as indicated in Section 2. Note the losses in epithelial intercellular E-cadherin and cytoskeletal keratin 18 in mutant p53 transfected cells. (B) Immune blotting shows that mutant p53 R175H prevents activation of the wt p53-activated p21WAF1 and PUMA, lowers the expression of E-cadherin and cytokeratin 18, and reciprocally increases in vimentin and proliferation-associated cyclin A. The results shown were representative of 2 different experiments. (C) Mutant p53 increases secretion of MMP-2. Similar results were seen with cells from different clones of each cell type.
testing the estrogenic response, adherent cells were extensively washed with colorless isotonic saline to remove serum, and were further incubated for 24 h in neutral red-free Dulbecco’ medium supplemented with 1.5% charcoal-treated estrogen-depleted serum and 1% neutral red-free Matrigel, including estradiol at the concentrations indicated in each experiment. Subsequently, cells were processed as previously indicated [15]. Cells showed no defined fluorescence after reaction with a negative control IgG in contrast to the reactivity seen with the specific monoclonal antibodies used.

2.8. Statistical studies

Standard deviations (S.D.) were used to determine a statistically significant difference in the median values shown for metabolic activity/cell viability and similar assays. These were carried out in quadruplicate and repeated at least 2 times. Generally, S.D. results usually were within ±5% with a 95% statistical significance (n = 4). The criterion for statistical significance was taken as p < 0.05 by Student’s t test, whenever indicated by *

3. Results

3.1. Mutant p53 R175H induces EMT, losses in p21WAF1, PUMA, cytokeratin 18 and secretion of MMP-2

Introducing the mutant p53 R175H into wt p53 MCF7 cells generated an ERα− cell line with both wt and mutated p53 proteins co-expressed [5,15]. Inactivation of the wt p53 allele by a mutant p53 possibly involves the interference of the latter with the wt p53 homotetramer needed for its transcriptional DNA binding activity [5]. Epithelial morphology was dramatically changed upon introduction of a mutant p53 R175H into wt p53 MCF-7 cells, concomitantly with an extensive loss of both E-cadherin and keratin 18, and the appearance of mesenchymal-looking cells (Fig. 1A). Immune blotting confirmed that cells expressing the mutant p53 R175H decreased expression of wt p53 regulated proteins PUMA and p21WAF1, seen in cells with p53 dysfunction [33,44]. This was paralleled by induction of mesenchymal vimentin [22] and proliferation-associated cyclin A [45], reciprocally with loss of cytokeratin 18 and E-cadherin (Fig. 1B). These changes correlated with greater secretion of the matrix-metalloprotease MMP-2 [34], preferentially evident in mutant p53 R175H (Fig. 1C).

3.2. Percentage of proliferating Ki67+/ERα− cells is susceptible to Bcl-2/Bcl-xL and is increased by mutant p53

Since co-expression of ERα− and the proliferation-associated marker Ki-67 occurs with ERα− breast cancer progression [2,3], and ERα can activate cell proliferation and the expression of anti-apoptotic genes like Bcl-2/Bcl-xL in ERα−positive breast cancer cells [35,36], laser scanning cytometry and immune blotting were used to show that ERα− expression is increased by mutant p53 R175H in MCF-7 cells, (Fig. 2A and B). Cytometric analysis of the co-expression of ERα− positive cells relative to Ki67− proliferating cells, revealed an increase in the % of proliferating Ki67+/ERα− mutant p53 cells (Fig. 2C and D). Moreover, exposure to a scrambled oligonucleotide sequence revealed 17.8% of Ki67−ERα− proliferating wt p53 cells compared to 34.4% Ki67−ERα− proliferating mutant p53 R175H cells (quadrant 2, Fig. 2E and F). In contrast, the % of Ki67+/ERα− co-expressing cells was diminished by the previously validated specific Bcl-2/Bcl-xL anti-sense oligonucleotide [38] in more than 50%, irrespective of p53 mutational status (quadrant2, Fig. 2D and E). No comparable

![Fig. 2. Mutant p53 R175H increases ERα expression and the % of ERα− cells coexpressing Ki67. (A and B) Expression of ERα was analyzed respectively by laser scanning cytometry and immune blotting. (C–F) Laser scanning cytometry was used to analyze co-expression of cells labeled with antibodies directed against ERα and proliferation-associated Ki67, in cells treated with scrambled or Bcl-2/Bcl-xL specific oligonucleotide sequences. Functional validation of these antisense oligonucleotides was previously demonstrated [37]. The results shown were similar in 2 different experiments.](image-url)
Mut p53 R175H decreases dependence on MEK activation inhibitor UO126 for cyclin A expression

Fig. 3. Mutant p53 R175H decreases dependence on estradiol and MEK inhibition for cyclin A expression. Laser scanning cytometry shows that proliferation associated cyclin A is preferentially increased in mutant p53 MCF-7 cells cultured in estrogen-depleted medium supplemented with the indicated estradiol concentrations, including 10 μM of MEK inhibitor UO126, whenever indicated. These results were consistently seen in 2 separate experiments.

3.3. Mut p53 R175H decreases dependence on estradiol and MEK activation for cyclin A expression

Inhibition of MEK/ERK activation using specific inhibitors is known to block DNA synthesis in wt p53 MCF-7 cells [26–30]. We now investigated by in situ laser scanning cytometry whether introducing mutant p53 R175H into MCF-7 cells modified their response to estrogen in the presence of UO126, a well-known MEK inhibitor [31], under estrogen depletion. This revealed 26.8% of proliferation-associated cyclin A in wt p53 MCF-7 cells exposed to 10 nM E2, and essentially background proliferation in the absence of exogenous E2 or when 10 nM E2 was supplemented with 10 μM UO126 (Fig. 3A, C and E). No comparable dependence was seen in mutant p53-expressing cells, which reproducibly expressed higher proliferation-associated cyclin A than their matched wt p53 counterparts (Fig. 3B, D and F).

3.4. 3-Bromopyruvate toxicity is preferential for mut p53 cells

Since proliferation leads to high glucose consumption, we compared the effect of the well known glycolytic inhibitor 2-deoxyglucose [40] with that of 3-bromopyruvate [41–43], in genetically-matched MCF-7 cells differing in their p53 status. Cells seeded for 20 h in complete medium with 10% serum and 20 mM glucose were transferred to glucose-free medium supplemented with 5 mM glucose and 5% dialyzed serum for 48 h, to measure cell viability [34]. This revealed selective toxicity of 75 μM 3-BrPA towards mutant p53 cells, with no comparable selectivity of 2.5 mM 2-deoxyglucose, which was inhibitory irrespective of p53 status similarly (Fig. 4A). Survival studies also showed preferential toxicity of 3-BrPA towards mutant p53 cells when assayed 5 days later (Fig. 4B).

3.5. p53 siRNA increases estradiol signaling, resistance to MEK inhibition, and susceptibility to 3-bromopyruvate in wt p53 MCF-7 cells

Although the consequences of p53 mutation are not identical to those of gene silencing [15], we carried out some assays in wt p53 MCF-7 cells in which p53 siRNA was introduced at high efficiency by electroporation to silence wt p53 expression compared to that seen in the same cells in which scrambled negative-control siRNA was similarly electroporated. Within 3 days of electroporation, cells were assayed by plating them in medium supplemented with estradiol-free serum and E2 concentrations indicated in each case,
to determine relative cell proliferation after 48 h. Parallel immune blotting revealed that p53 siRNA decreased p53 protein expression when normalized to reference actin levels (Fig. 5A). Relative cell proliferation independently of MEK inhibition with UO126 or exogenous estradiol was preferentially decreased by p53 silencing (Fig. 5B and C). Reciprocally, loss of cell proliferation by 3-bromopyruvate (3-BrPA) was increased by p53 siRNA (Fig. 5D). Taken together, these observations are compatible with the results shown in Figs. 3 and 4, implying that p53 inactivation by mutation or silencing decreases dependence on exogenous estradiol and MEK inhibition, but confers susceptibility to 3-BrPA in MCF-7 ERα− breast carcinoma cells.

4. Discussion

EMT is important in breast cancer progression, but its diverse consequences on autocrine proliferation and glycolysis are incompletely understood. Using different isolates of genetically-matched breast cancer MCF-7 cells with unequal p53 genotype [15], we found that transducing mutant p53 R175H into ERα− breast cancer MCF-7 cells, induces EMT and proliferation in response to restrictive amounts of estrogen. This correlated with higher estrogen receptor alpha (ERα) expression and increased co-expression of the estrogen receptor ERα and the proliferation-associated marker Ki-67 compared to their matched wt TP53 counterparts. We also showed that a previously validated Bcl-2/Bcl-xL bispecific antisense oligonucleotide [37] decreased the ERα+/Ki67+ proliferating population, irrespective of their p53 status in agreement with studies demonstrating that ERα can activate proliferation and expression of anti-apoptotic genes like Bcl-2 in mutant p53 MCF-7 cells [35,36]. Moreover, both Bcl-2 and Bcl-xL are known to be important in regulating cell proliferation through their effects on glucose metabolism [46]. Our findings also imply that p53 inactivation in MCF-7 cells reported to increase EGFR-R activation [15], also promotes adaptive hypersensitivity to estradiol [46], supporting the link between EGFR/ERK activation and ERα [36]. This interpretation is compatible with results reporting that greater ERα expression and germine disruption of one p53 allele increased extracellular signal-regulated kinase 1/2 (ERK1/2) and female breast cancer risk [11]. Since phosphorylation at serines 104 and 106 by Erk1/2/MAPK is important for ERα activity [29] and this pathway mediates response to anti-estrogens [26–30], we also investigated whether the decreased dependence on estrogen for cell proliferation induced by mutant p53R175H also involved resistance to MEK inhibitors like UO126 [31]. It was indeed seen that mutant p53 R175H induces MEK-independent proliferation. Whereas expression of proliferation-associated cyclin A [45] was dependent on estradiol and inhibited by the MEK inhibitor UO126 in wt p53 cells [31], these restrictions did not occur in cells with mutant p53 R175H, which showed higher proliferation-associated cyclin A in the presence of the MEK inhibitor and with low estradiol, compared to those with wt p53 (Fig. 3). Mutant p53 R175H cells which undergo EMT and proliferate irrespective of low estrogen and chemical MEK inhibition are likely to consume more glucose to support estrogen modulated energy consumption [38,39]. Nevertheless, it was
noteworthy that milimolar levels of 2-deoxyglucose [40] exerted a comparable inhibitory effect against MCF-7 cells irrespective of their p53 status. This was in contrast to the higher and preferential toxicity of micromolar concentrations of the pyruvate analog 3-BrPA [43] towards mutant p53 MCF-7 cells (Fig. 4). Moreover, p53 siRNA electroporation which causes transient p53 silencing promoted increased proliferation in response to lower estradiol and greater susceptibility to 3-BrPA, supporting the assertion that

Fig. 5. p53 siRNA electroporation increases estradiol signaling resistance to MEK inhibition, and 3-BrPA sensitivity in wt p53 MCF-7 cells. Cells electroporated with p53 siRNA or negative control sequences were assayed for: (A) changes in p53 expression normalized to actin by immune blotting; (B) decreased dependence on MEK inhibition with 5 μM U0126 for proliferation measured by Alamar Blue fluorescence; (C) enhanced response to estradiol (E2) measured by Alamar Blue fluorescence and (D) relative survival in response to 3-BrPA evidenced by Alamar Blue. These results shown are representative of 2 different experiments, in which (B) and (C) were carried out at n = 8.

Fig. 6. Decreased dependence on estradiol, and MEK inhibition and EMT induced by mutant p53 R175H confer greater susceptibility to 3-bromopyruvate in ERα+ breast cancer cells. (Left) Breast carcinoma MCF-7 cells transduced with empty vector exhibit wt p53 function and epithelial organization mediated by intercellular E-cadherin and cytoskeletal keratin 18. (Right) Stable transduction of a R175H p53 mutation causes epithelial disorganization, and EMT in MCF-7 cells. This results in lower E-cadherin and keratin 18. Functional consequences of p53 dysfunction are proliferation with diminished dependence on estrogen and MEK inhibition, and greater susceptibility to 3-bromopyruvate.
abrogation of p53 function in ERα* breast cancer cells increases estrogen signaling and susceptibility to 3-BrPA. The greater efficacy of 3-BrPA against ERα* MCF-7 cells with p53 inactivation compared to that of 2-deoxyglucose may be due to the fact that this pyruvate antagonist not only is capable of inhibiting both glycolysis and mitochondrial oxidative phosphorylation [41–43], but also can act as an anti-oxidant [47], in contrast to 2-deoxyglucose which preferentially acts as a hexokinase inhibitor of glycolysis [40].

Our Fig. 6 summary and our overall results suggests that mutant p53 R175H or p53 dysfunction might antagonize response to anti-estrogenic therapy, by lowering the threshold of estrogen required for growth. This may occur because mutant p53 could help to counteract proapoptosomal degradation of ERα* and indirectly increase expression of this receptor [48]. Another consequence of the p53 R175H mutation on ERα* breast cancer cells is the diminished ability of the MEK inhibitor, U0126 [31,49] to counteract cell proliferation. Our results suppressing wt p53 function by mutation or silencing in MCF-7 cells which is one of the best pre-clinical models for TP53/ERα* breast cancer cells, are compatible with data from breast cancer patients showing persistent activation of the MAPK pathway with diminished response to tamoxifen but not chemotherapy [27,50,51]. Taken together, our results support the rational of testing ERα* breast cancer for p53 mutation to prevent resistance to selective estrogen receptor modulator (SERM) treatments [27,50–52]. Translational research recently demonstrated that p53 dysfunction and active estrogen receptor (ER) signalling significantly influence survival and are prognostically most relevant in ERα* breast cancers [27,50–53]. This report implies that 3-BrPA or similar molecules capable of targeting both glycolysis and mitochondrial respiration may be helpful as adjuvants in endocrine therapy against ERα* breast cancers with inactivated p53.

This research was carried out in vitro with cultured breast cancer cells and did not require approval from our bioethical committee. Hence, this study is in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Mary Strasberg-Rieber contributed fundamentally to the acquisition, design and interpretation of data, and to the critical revision of the manuscript. Manuel Rieber conceived and designed acquisition of data, and was involved in drafting and critically revising the manuscript. All authors read and approved the final manuscript.

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