Novel roles of 1α,25(OH)$_2$D$_3$ on DNA repair provide new strategies for breast cancer treatment

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Abstract

Breast cancers classified as triple-negative (TNBC) and BRCA1-deficient, are particularly aggressive and difficult to treat. A major breakthrough was the finding that these tumors are exquisitely sensitive to inhibitors of poly(ADP-ribose) polymerase (PARPi). Phase II clinical trials have shown encouraging outcomes, with tolerable side effects. However, a significant fraction of these cancers acquire resistance. Elegant studies demonstrated that loss of the DNA repair protein 53BP1 contributes to the resistance of BRCA1-deficient cells and tumors to PARPi. Thus, raising the levels of 53BP1 in these aggressive tumors could potentially restore their sensitivity to PARPi and other genotoxic agents. We will review here our studies revealing that 1α,25(OH)$_2$D$_3$, an active form of vitamin D, stabilizes 53BP1 levels in tumor cells. Breast tumor cells that become BRCA1-deficient activate cathepsin L-mediated degradation of 53BP1 to ensure genome stability and proliferation. Importantly, 1α,25(OH)$_2$D$_3$ treatment restores the levels of 53BP1 as efficiently as cathepsin L inhibitors, which results in increased genomic instability in response to PARPi or radiation, and reduced proliferation. Furthermore, analysis of human breast tumors identified nuclear cathepsin L as a positive biomarker for TNBC, which correlates inversely with 53BP1 when vitamin D receptor (VDR) nuclear levels are low. The major findings of these studies are: 1) identification of a new pathway contributing to breast cancers with the poorest prognosis; 2) discovery of the ability of 1α,25(OH)$_2$D$_3$ to inhibit this pathway; and 3) discovery of a triple biomarker signature for identification of patients that could benefit from the treatment.

Keywords

BRCA1; 53BP1; cathepsin L; DNA repair; vitamin D; 1α,25(OH)$_2$D$_3$; breast cancer

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Introduction

Breast cancer is the leading cause of cancer death in women worldwide (Jemal, Center et al. 2010). Women carrying germline mutations in BRCA1 have a 50–80% risk of developing breast cancer during their lifetime (King et al. 2003). The tumors that arise are highly invasive, tend to lack expression of estrogen and progesterone receptors, and do not show upregulation of HER2, being classified as "triple negative breast cancers" (TNBC) (Sorlie et al. 2003; Turner and Reis-Filho 2006). Interestingly, a subset of sporadic TNBC present with DNA repair defects and gene expression profiles that phenocopy BRCA1 related cancers and as such, are often responsive to therapeutic strategies that exploit DNA repair deficiencies (Foulkes, Smith et al. 2010).

A major breakthrough in the treatment of BRCA1-deficient and TNBC patients was the finding that these tumors are exquisitely sensitive to poly(ADP-ribose) polymerase inhibitors (PARPi) (Farmer, McCabe et al. 2005; Helleday, Bryant et al. 2005). Inhibition of PARP1 and the closely related PARP2 proteins (Satoh and Lindahl 1992; Ame, Rolli et al. 1999; Allinson, Dianova et al. 2003; Woodhouse and Dianov 2008) hinders single-strand breaks (SSBs) repair, which in turn leads to the stalling of the replication fork and the formation of double-strand breaks (DSBs) that need to be repaired primarily by homologous recombination (HR) (Helleday, Bryant et al. 2005; Ashworth 2008). BRCA1 plays a critical role in the repair of DNA DSBs by HR (Scully, Ganesan et al. 1996; Scully and Livingston 2000), a process that utilizes sister chromatids as templates for recombination resulting in error-free DNA DSBs repair. Therefore, BRCA1-deficient cells cannot deal with the amount of DSBs generated by PARPi, resulting in proliferation arrest and cell death. The demonstrated vulnerability of BRCA1-deficient cells and tumors to PARPi has expedited their use in the clinic (Rottenberg, Jaspers et al. 2008; Fong, Boss et al. 2009; Audeh, Carmichael et al. 2010). Phase II studies with PARPi have shown a significant response rate in women carrying BRCA1 mutations, with tolerable side effects (Tutt, Robson et al. 2010). Thus, the use of PARPi as single agents or in combination with radiation and chemotherapy represents a leading strategy for the management of breast cancers, especially BRCA1-deficient tumors. However, a significant fraction of these cancers acquire resistance to PARPi, stressing the importance of understanding the molecular mechanisms behind resistance, which will allow the design of novel therapeutic strategies.

Recent ground-breaking studies demonstrated that loss of 53BP1 is "synthetically viable" with BRCA1 loss (Aly and Ganesan 2011). Loss of 53BP1 promotes the viability of BRCA1-deficient cells by rescuing some of the phenotypes associated with the loss of BRCA1 function in HR (Cao, Xu et al. 2009; Bothmer, Robbiani et al. 2010; Bouwman, Aly et al. 2010; Bunting, Callen et al. 2010. Most importantly, loss of 53BP1 induces resistance of BRCA1-deficient cells to PARPi (Jaspers, Kersbergen et al. 2013). 53BP1 facilitates the repair of DNA DSBs by the error prone NHEJ mechanism (Schultz, Chehab et al. 2000; Fernandez-Capetillo, Chen et al. 2002; Wang, Matsuoka et al. 2002; Xie, Hartlerode et al. 2007; Difilippantonio, Gapud et al. 2008; Dimitrova, Chen et al. 2008). The current view is that loss of BRCA1 results in defective end-resection of DNA DSBs, an essential event in HR. Accumulation of 53BP1 at the breaks in this context promotes massive NHEJ with the consequent genomic instability that causes proliferation arrest. However, in cells double

deficient in BRCA1 and 53BP1 end-resection is allowed, rescuing at least partially, the HR repair mechanism. As a consequence, these cells exhibit a much lesser degree of genomic instability resulting in increased survival and are less sensitive to PARPi. Altogether these studies indicate that loss of 53BP1 in the context of BRCA1 deficiency is critical for breast tumor progression. As such, loss of 53BP1 negatively correlates with a greater likelihood of metastases and significant decreased survival (Bouwman, Aly et al. 2010; Li, Xu et al. 2012). Thus, upregulation of 53BP1 levels represents a promising strategy to prevent progression of breast tumors with the poorest prognosis as well as to improve their response to PARPi and other DNA damaging strategies such as radiation. However, a limiting factor in the progress towards this goal is the lack of information about how the levels of 53BP1 are regulated in normal cells or downregulated in tumor cells. Here, we review the studies that led us to the identification of a new pathway regulating the levels of 53BP1 protein in mammalian cells, and how this pathway is activated in different disease states. Lastly, we will discuss the possible significance of this novel pathway for diagnosis and design of cancer therapies.

Studies in A-type lamins-deficient cells reveal a new pathway regulating 53BP1

Our initial studies in A-type lamins-deficient mouse embryonic fibroblasts (*Lmna*$^{-/-}$ MEFs) and lamins A/C-depleted human and mouse cells, revealed a marked decrease in the levels of 53BP1, which was accompanied by defects in DNA repair by NHEJ (Gonzalez-Suarez, Redwood et al. 2009; Gonzalez-Suarez, Redwood et al. 2009; Redwood, Perkins et al. 2011). In addition, we found that loss of A-type lamins results in upregulation of the cysteine protease cathepsin L (CTSL), and its accumulation in the nucleus (Gonzalez-Suarez, Redwood et al. 2011). We also demonstrated that the increase in CTSL, a hallmark of numerous cancers (Lankelma, Voorend et al. 2010), is responsible for the degradation of 53BP1 in lamins A/C-deficient cells. Accordingly, depletion of CTSL rescued normal levels of 53BP1 in these cells, as well as their defects in NHEJ (Gonzalez-Suarez, Redwood et al. 2011) (Figure 1).

Given the role of CTSL in regulating 53BP1 stability, we tested whether inhibition of CTSL activity could restore the levels of 53BP1 in A-type lamins-deficient cells, since such strategy could be relevant for therapy. We found that treatment with the specific CTSL inhibitor Z-FY-CHO restored the levels of 53BP1 in these cells (Gonzalez-Suarez, Redwood et al. 2011). Interestingly, we also found that $1\alpha,25(OH)_2D_3$ (the active form of vitamin D), via inhibition of CTSL activity, stabilized 53BP1 protein levels. Although the molecular mechanism by which $1\alpha,25(OH)_2D_3$ inhibits CTSL activity is poorly understood, some studies have indicated that the effect might be mediated by cystatins, endogenous inhibitors of cathepsins. In particular, a study in human colon cancer cells demonstrated that $1\alpha,25(OH)_2D_3$, via the vitamin D receptor (VDR), upregulates the levels of cystatin D, which in turn inhibits the activity of cathepsins, including CTSL (Alvarez-Diaz, Valle et al. 2009). Our gene promoter analysis identified mouse cystatin B as a gene containing at least two VDR/RXR heterodimer binding sites. In addition, we found that cystatin B is downregulated

in lamins A/C-deficient cells, thus suggesting that the effect of $1\alpha,25(OH)_2D_3$ could be mediated by cystatins (Figure 1).

Overall, the studies in A-type lamins-deficient cells allowed us to identify a new pathway responsible for regulating the stability of 53BP1 protein in mammalian cells (CTSL-mediated degradation), and a way to inhibit this pathway with therapeutic purposes (CTSL inhibition via specific inhibitors or $1\alpha,25(OH)_2D_3$). Importantly, the effect of CTSL on 53BP1, and as a consequence on DNA repair, is not exclusive of A-type lamins-deficient cells, as overexpression of CTSL in wild-type MEFs results in degradation of 53BP1 and defective NHEJ (Gonzalez-Suarez, Redwood et al. 2011). This suggests that tumors in which CTSL is upregulated could present with genomic instability due to loss of 53BP1, in addition to the characteristic effects of CTSL overexpression on the degradation of the extracellular matrix (Lankelma, Voorend et al. 2010).

Additional studies revealed that loss of A-type lamins results in a marked decrease in the levels of BRCA1 and RAD51 transcripts and proteins (Redwood, Perkins et al. 2011) (Figure 1). As a consequence, these cells present with a 40% reduction in DNA repair by HR. Whether or not $1\alpha,25(OH)_2D_3$ or cathepsin inhibitors are able to rescue the levels of BRCA1 and RAD51 remains unanswered. However, the fact that $1\alpha,25(OH)_2D_3$ treatment reduced the basal levels of unrepaired DNA damage and the nuclear morphological abnormalities that characterize lamins A/C-deficient cells suggests that this is a possibility. Consistent with this possibility, a previous study showed that $1\alpha,25(OH)_2D_3$ treatment results in VDR-mediated induction of BRCA1 gene expression (Campbell, Gombart et al. 2000). Future experiments are needed to test the effect of $1\alpha,25(OH)_2D_3$ treatment on the expression of BRCA1 and RAD51 in lamins A/C-deficient cells, which could provide important clues for therapy.

Mechanisms behind downregulation of 53BP1 in BRCA1-deficient cells

Loss of BRCA1 results in profound genomic instability and proliferation arrest, in part due to inhibition of HR. Thus, an intriguing question in the field has been how BRCA1-deficient tumor cells are able to survive in the context of such profound genomic instability. Loss of 53BP1 seems to play a major role in the survival and proliferation of BRCA1-deficient cells (Cao, Xu et al. 2009; Bothmer, Robbiani et al. 2010; Bouwman, Aly et al. 2010; Bunting, Callen et al. 2010). Thus, understanding the mechanisms by which 53BP1 is lost in cancer cells could provide new therapeutic targets.

Based on the data obtained in A-type lamins-deficient cells, we hypothesized that BRCA1-deficient breast cancer cells might activate CTSL-mediated degradation of 53BP1 as a means to ensure viability and proliferation. If our hypothesis were correct, we could use $1\alpha,25(OH)_2D_3$ or cathepsin inhibitors to increase 53BP1 levels with therapeutic purposes.

To test our hypothesis, we depleted BRCA1 with a specific shRNAs in MCF7 cells, breast tumor cells that are proficient in both BRCA1 and 53BP1. As expected, depletion of BRCA1 leads to a proliferation arrest, which was accompanied by an increase in genomic instability (Grotsky, Gonzalez-Suarez et al. 2013). Growth arrested cells exhibited normal levels of 53BP1 and CTSL immediately after depletion of BRCA1. However, after

approximately 2 weeks, BRCA1-deficient cells resumed proliferation, although at a lower rate than control cells. Interestingly, cells that overcome the growth arrest referred to as BOGA cells (BRCA1-deficient cells that Overcome Growth Arrest) exhibit decreased 53BP1 levels and increased CTSL levels, while still maintaining the depletion of BRCA1 (Figure 2). Monitoring transcripts levels revealed that CTSL is upregulated transcriptionally in these cells, and that 53BP1 transcripts levels are not decreased, indicating a decrease in 53BP1 protein stability, similarly to what we observed in A-type lamins-deficient cells.

To determine if the loss of 53BP1 is responsible for the bypass of growth arrest we depleted 53BP1 via specific shRNAs prior to depletion of BRCA1. Importantly, we found that while cells depleted only of BRCA1 undergo growth arrest, cells depleted of 53BP1 continue proliferating after depletion of BRCA1. These results demonstrate that 53BP1 plays a major role in the growth arrest induced by BRCA1 depletion (Grotsky, Gonzalez-Suarez et al. 2013).

Next, we determined if CTSL is responsible for the degradation of 53BP1 following depletion of BRCA1. We performed acute depletion of CTSL in control and BOGA cells. Importantly, depletion of CTSL stabilized 53BP1 protein levels in BOGA cells mirroring those of control cells. In addition, we inhibited CTSL activity in control and BOGA cells via treatment with $1\alpha,25(OH)_2D_3$ or the broad cathepsin inhibitor E64. In both cases, inhibition of CTSL activity leads to stabilization of 53BP1. These data demonstrate that cells growth arrested following depletion of BRCA1 activate CTSL-mediated degradation of 53BP1, allowing cells to bypass growth arrest (Figure 2). In addition, inhibition of CTSL could be used to increase 53BP1 levels in the context of BRCA1 deficiency.

Consequences of stabilization of 53BP1 in BRCA1-deficient cells

The generation of DNA DSBs by ionizing radiation (IR) leads to activation of the DNA damage response (DDR), a complex pathway that senses, signals, and ultimately repairs the DNA lesions generated (Zhou and Elledge 2000; Khanna and Jackson 2001; Hoeijmakers 2009; Jackson and Bartek 2009; Ciccia and Elledge 2010). Activation of DDR leads to the recruitment of a whole variety of factors to the site of damage, which can be easily visualized as nuclear foci by immunofluorescence with specific antibodies, and are referred to as ionizing radiation-induced foci (IRIF). 53BP1 and BRCA1 are recruited to DNA DSBs shortly after radiation (Figure 3). 53BP1 binding facilitates the recruitment of the NHEJ repair machinery (Wyman and Kanaar 2006; Shibata, Conrad et al. 2011), while inhibiting HR. In contrast, recruitment of BRCA1 is associated with repair by HR. BRCA1 facilitates recruitment of RAD51, a protein that is essential for homology search and strand invasion during recombination, and that is commonly used as a readout of HR (Scully, Chen et al. 1997; Scully, Chen et al. 1997; Moynahan, Chiu et al. 1999; Snouwaert, Gowen et al. 1999; Schlegel, Jodelka et al. 2006).

To determine the ability of BOGA cells to repair IR-induced DSBs by NHEJ and HR, we monitored the recruitment of 53BP1 and RAD51 to sites of damage. As expected, BOGA cells were unable to form BRCA1 or 53BP1 IRIF, consistent with the decreased protein levels (Grotsky, Gonzalez-Suarez et al. 2013). In contrast, we found that RAD51 IRIF

readily formed in BOGA cells, although their time of retention at the DSBs was reduced. This is consistent with previous studies showing that loss of both BRCA1 and 53BP1 restores at least partially the ability of cells to repair DSBs by HR (Bunting, Callen et al. 2010). Importantly, we found that formation of 53BP1 IRIF was rescued in BOGA cells by inhibiting CTSL activity via $1\alpha,25(OH)_2D_3$ treatment. These results indicate that inhibition of CTSL by $1\alpha,25(OH)_2D_3$ not only rescues the levels of 53BP1, but also its ability to be recruited to sites of DNA damage. Interestingly, treatment with $1\alpha,25(OH)_2D_3$ or depletion of CTSL partially reduced the formation of RAD51 IRIF in BOGA cells. Altogether, these studies reveal an unprecedented role for $1\alpha,25(OH)_2D_3$ in regulating the two main pathways of DNA DSBs repair, NHEJ and HR, especially in tumor cells that are deficient in BRCA1 and 53BP1, as is the case of some TNBC and BRCA1-related tumors. Stabilization of 53BP1 by $1\alpha,25(OH)_2D_3$ seems to promote NHEJ and inhibit HR. Given that HR-deficient cells are sensitivite to PARPi and other DNA damaging strategies, $1\alpha,25(OH)_2D_3$ treatment could potentially improve the sensitivity of BRCA1-deficient cells that become resistant to these compounds due to the loss of 53BP1, as discussed below.

Vitamin D as a new strategy to induce genomic instability and growth arrest

Based on the previous data, we hypothesized that stabilization of 53BP1 in the context of BRCA1 depletion could hinder the ability of these cells to deal with IR-induced damage, leading to genomic instability and cell death. To test this hypothesis, control and BOGA cells were treated with $1\alpha,25(OH)_2D_3$ in order to stabilize 53BP1 twenty-four hours prior to irradiation. Metaphase spreads analysis revealed that both, control cells and BOGA cells were able to repair breaks induced by IR, and did not present with much genomic instability. In contrast, treatment with $1\alpha,25(OH)_2D_3$ or with the cathepsin inhibitor E64 increased profoundly the extent of chromosomal aberrations in BOGA cells, but not in control cells. Both, $1\alpha,25(OH)_2D_3$ and cathepsin inhibitors are able to increase genomic instability in response to IR (Grotsky, Gonzalez-Suarez et al. 2013). In addition, these treatments increased radiosensitization, as shown by a decrease in proliferation with the combined treatment. These results provide a possible strategy to induce radiosensitization in BRCA1-deficient cells that activate CTSL-mediated degradation of 53BP1. Furthermore, stabilization of 53BP1 by $1\alpha,25(OH)_2D_3$ results in increased genomic instability in response to PARPi. These results indicate that $1\alpha,25(OH)_2D_3$ or cathepsin inhibitors could be used in combination with radiation or PARPi to reduce the growth of tumors that have activated CTSL-mediated degradation of 53BP1, if these tumors could be identified.

Activation of CTSL-mediated degradation of 53BP1 in TNBC

A key question raised by our findings was whether activation of CTSL-mediated degradation of 53BP1 is observed in human breast tumors. In collaboration with the groups of Adriana Dusso and Xavier Matias-Guiu in the University of Lleida, Spain, we performed immunohistochemical (IHC) analysis of a breast tumor tissue microarray constructed from biopsies of different molecular types to monitor the levels of CTSL and 53BP1 (Grotsky, Gonzalez-Suarez et al. 2013). A total of 249 sporadic tumors classified in four different types were analyzed: Luminal A, Luminal B, HER2 and TN. We found that all tumor types

present with high levels of cytoplasmic CTSL. However, a subset of TNBC (60%) exhibits high levels of nuclear CTSL, in contrast to approximately 30% of all other molecular types. We also confirmed that a high percentage of TNBC (75%) have low levels of 53BP1, as previously reported (Bouwman, Aly et al. 2010). Thus, we identified nuclear CTSL as a novel biomarker for TNBC tumors, and a correlation between high nuclear CTSL and low 53BP1 levels in these tumors. These results suggest that CTSL-mediated degradation of 53BP1 could be one of the mechanisms responsible for the observed loss of this DNA repair factor in TNBC. However, not all TNBC exhibited increased CTSL and decreased 53BP1. In some tumors, we observed high levels of both CTSL and 53BP1, suggesting that additional factors could be regulating the ability of CTSL to degrade 53BP1 in these tumors. Identifying these factors could help to discriminate subsets of patients in which this pathway is activated.

Previous studies in human colon cancer cells found a correlation between levels of VDR and expression of cystatin D, an endogenous inhibitor of several cathepsins including CTSL (Alvarez-Diaz, Valle et al. 2009; Alvarez-Diaz, Larriba et al. 2010). They also showed upregulation of cystatin D by $1\alpha,25(OH)_2D_3$. In addition, our data in BOGA cells show that $1\alpha,25(OH)_2D_3$ inhibits CTSL-mediated degradation of 53BP1 (Grotsky, Gonzalez-Suarez et al. 2013), and most $1\alpha,25(OH)_2D_3$ actions require a functional nuclear VDR (Dusso, Brown et al. 2005). Thus, we hypothesized that an increase in nuclear VDR might lead to activation of cystatins and inhibition of CTSL-mediated degradation of 53BP1. High levels of nuclear VDR could explain the signature of tumors with high levels of both nuclear CTSL and 53BP1. To test this model, we performed IHC analysis of nuclear VDR in all 249 sporadic tumors. Then, we analyzed the linear relationship between 53BP1 and CTSL for those tumors with low nuclear VDR expression (below the median). We found that in 30% of all 249 tumors with low VDR expression, the decrease in 53BP1 can be account for by the increase in nuclear CTSL. This effect is even more striking if we consider only TNBC. In this case, 80% of the TNBC with low nuclear VDR, the decrease in 53BP1 can be accounted for by the increase in nuclear CTSL. This represents a strong correlation between levels of nuclear VDR and the ability of CTSL to decrease 53BP1 levels.

Thus, we have identified a novel triple nuclear biomarker signature to stratify TNBC patients. We find two signatures in these patients. One of the signatures shows high nuclear CTSL, high 53BP1 and high VDR. We hypothesize that the high levels of VDR are inhibiting CTSL-mediated degradation of 53BP1, thus the pathway is turned off. A second signature shows high CTSL, low 53BP1 and low VDR, thus suggesting that CTSL-mediated degradation of 53BP1 is turned on. We envision that these signatures could potentially have predictive value for drug response, such that patients with signature 1 might respond to PARPi, due to the high levels of 53BP1. In contrast, patients with signature 2 might be resistant to PARPi due to the loss of 53BP1. These patients might benefit from inhibition of CTSL-mediated degradation of 53BP1 via treatment with $1\alpha,25(OH)_2D_3$ or cathepsin inhibitors.

A new biomarker signature for BRCA1-related tumors

Given our findings that loss of BRCA1 activates CTSL-mediated degradation of 53BP1, we tested whether increased CTSL is observed in patients with breast cancer that carry germline mutations in BRCA1 or BRCA2. We found increased levels of nuclear CTSL in BRCA1-related tumors, but not in BRCA2 tumors. Similarly, we observed a marked decrease in 53BP1 levels in BRCA1 tumors when compared to BRCA2. Interestingly, BRCA1-related tumors exhibit lower levels of nuclear VDR than BRCA2-related tumors. Specifically, 85% of patients with BRCA1 mutations exhibit nuclear CTSL levels over the median, versus 53% of patients with BRCA2 mutations. Accordingly, 88% of patients with BRCA1 mutations present with 53BP1 levels below the median versus 15% of BRCA2 patients. Lastly, 83% of BRCA1 patients present with nuclear VDR levels under the median. These results in breast cancer patients support our model that loss of BRCA1 leads to activation of CTSL-mediated degradation of 53BP1, and that $1\alpha,25(OH)_2D_3$ via activation of VDR, can inactivate this pathway.

Summary

Our studies showed that depletion of BRCA1 leads to growth arrest due to defects in HR and increased genomic instability. BRCA1-deficient cells can activate compensatory mechanisms that allow them to overcome genomic instability and growth arrest. We found that one of these mechanisms is the activation of CTSL-mediated degradation of 53BP1. Importantly, inhibition of CTSL-mediated degradation of 53BP1 via increased levels of VDR or treatment with $1\alpha,25(OH)_2D_3$ or cathepsin inhibitors results in HR defects, genomic instability, and increased sensitivity to DNA damaging therapeutic strategies, such as IR or PARPi. In addition, we identified a strong inverse linear correlation between nuclear CTSL and 53BP1 levels in BRCA1-deficient tumors and TNBC that present with low levels of nuclear VDR. This novel triple biomarker signature could potentially have predictive value for drug response, as it could allow the identification of breast cancer patients that activate CTSL-mediated degradation of 53BP1. These patients could benefit from a therapeutic strategy such as $1\alpha,25(OH)_2D_3$ or cathepsin inhibitors to stabilize 53BP1 and restore sensitivity to PARPi or radiation. Although preclinical studies in mice are needed to evaluate the effect of inhibition of CTSL activity in the progression of specific types of breast cancers, $1\alpha,25(OH)_2D_3$ and cathepsin inhibitors have emerged in these studies as new possible therapeutic strategies for breast cancers with the poorest prognosis, such is the case of TNBC and BRCA1-related tumors.

The major findings of these studies are: 1) identification of a new pathway -CTSL-mediated degradation of 53BP1- contributing to breast cancers with the poorest prognosis; 2) discovery of the ability of $1\alpha,25(OH)_2D_3$ to inhibit this pathway; and 3) discovery of a triple biomarker signature -nuclear levels of VDR, CTSL and 53BP1- for the identification of patients that could benefit from the treatment.

Abbreviations list

TNBC   triple negative breast cancers

BRCA1	breast cancer susceptibility gene 1
PARPi	poly(ADP-ribose) polymerase inhibitors
CTSL	cathepsin L
DSBs	double strand breaks
HR	homologous recombination
NHEJ	non homologous end joining
VDR	vitamin D receptor
IRIF	ionizing radiation induced foci

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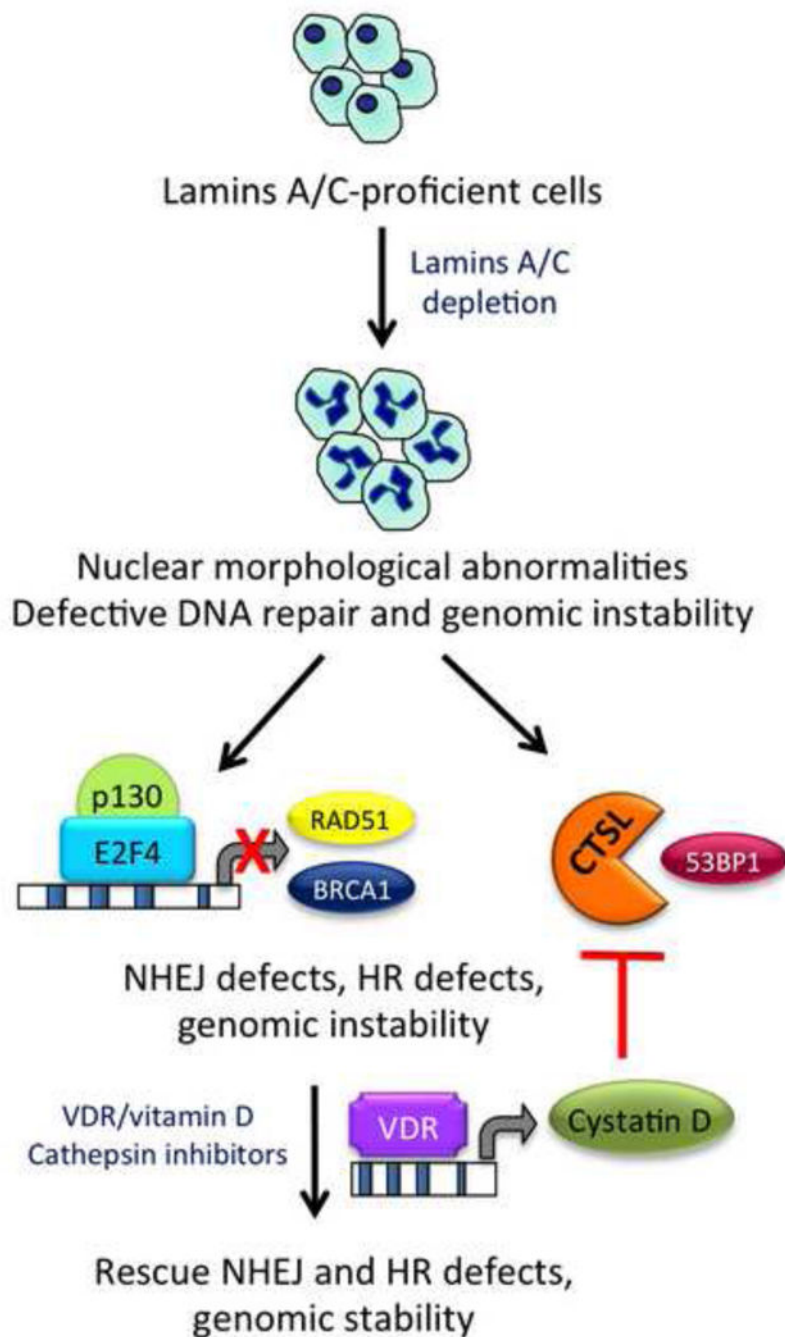
Highlights

Loss of 53BP1 in tumors leads to therapy resistance.

Identified a new pathway responsible for 53BP1 loss in breast cancers with poor prognosis.

Discovered a way to inhibit 53BP1 loss (vitamin D treatment).

Identified a triple nuclear biomarker signature to classify patients with pathway active that could benefit from treatment.

Figure 1. Model of mechanisms contributing to genomic instability in lamins-deficient cells
A-type lamins play a key role in the maintenance of nuclear architecture and genome
integrity. Loss of A-type lamins results in profound nuclear morphological abnormalities,
defects in DNA repair, and genomic instability. The figure illustrates some of the
mechanisms behind DNA repair deficiencies, including activation of CTSL-mediated
degradation of 53BP1 protein, and transcriptional downregulation of HR proteins BRCA1
and RAD51. A repressor complex formed by the Rb family member p130 and the
transcription factor E2F4 participates in the repression. As a consequence, the two main

mechanisms of DNA DSB repair -NHEJ and HR- are hindered, resulting in genomic instability. Importantly, inhibition of CTSL via vitamin D treatment restores 53BP1 protein levels as well as unrepaired DNA damage in lamins-deficient cells. Although the mechanism by which vitamin D inhibits CTSL is still under investigation, some data suggests a role for cystatin D mediating the effect.
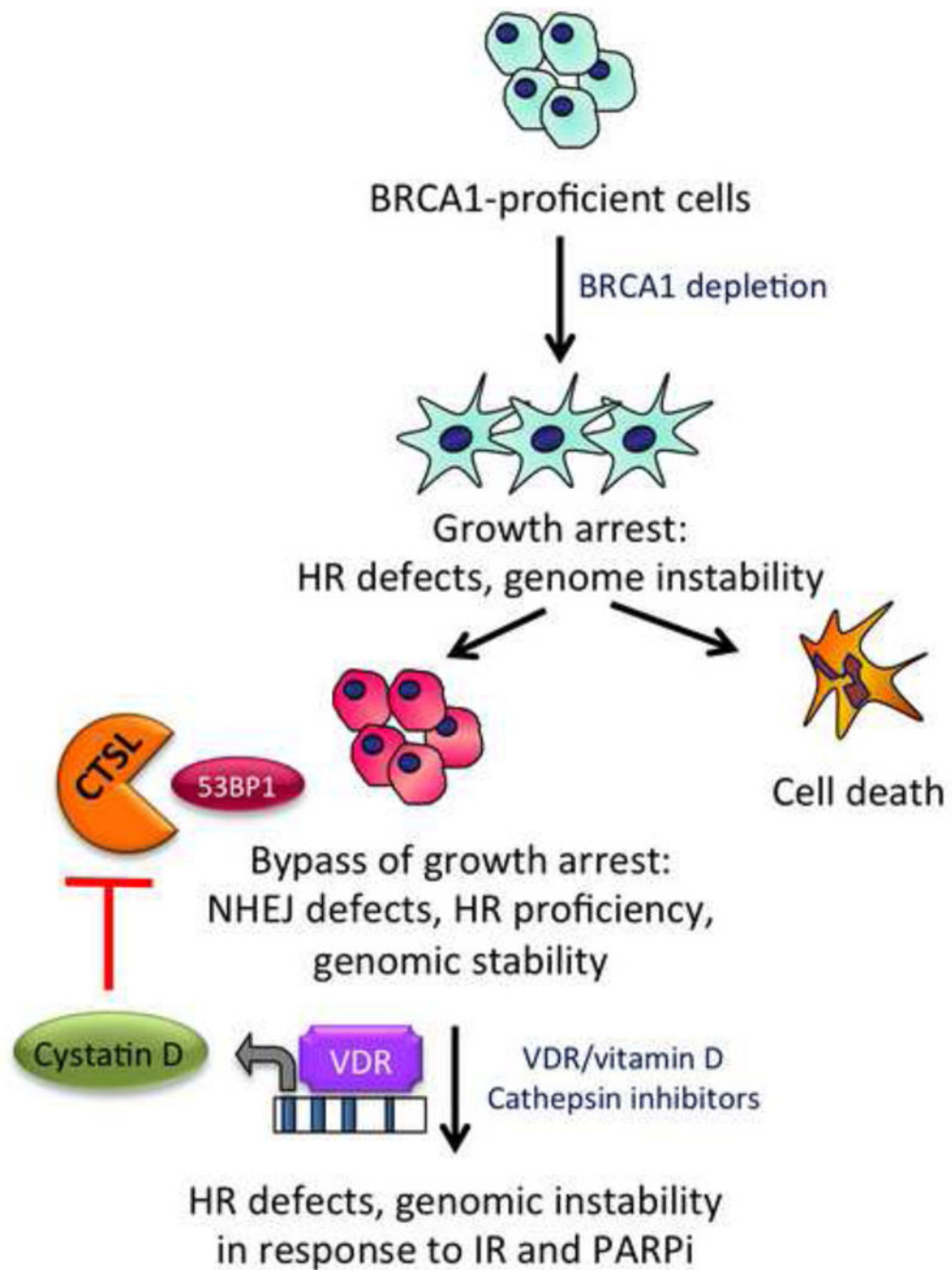
Figure 2. Model of mechanisms contributing to the bypass of growth arrest in BRCA1-deficient cells

Loss of BRCA1 causes profound genomic instability due to defects in HR, and proliferation arrest. However, after some time in culture, BRCA1-deficient cells activate some mechanisms that allow them to overcome genomic instability and growth arrest. One of these mechanisms is CTSL-mediated degradation of 53BP1. Thus, inhibition of CTSL and stabilization of 53BP1 via treatment with vitamin D or cathepsin inhibitors causes genomic instability and decreased proliferation and survival in the context of BRCA1-deficiency.

Furthermore, our model predicts that nuclear VDR regulates the identified mechanism. This provides a new possible therapeutic strategy for BRCA1-related cancers and TNBC.
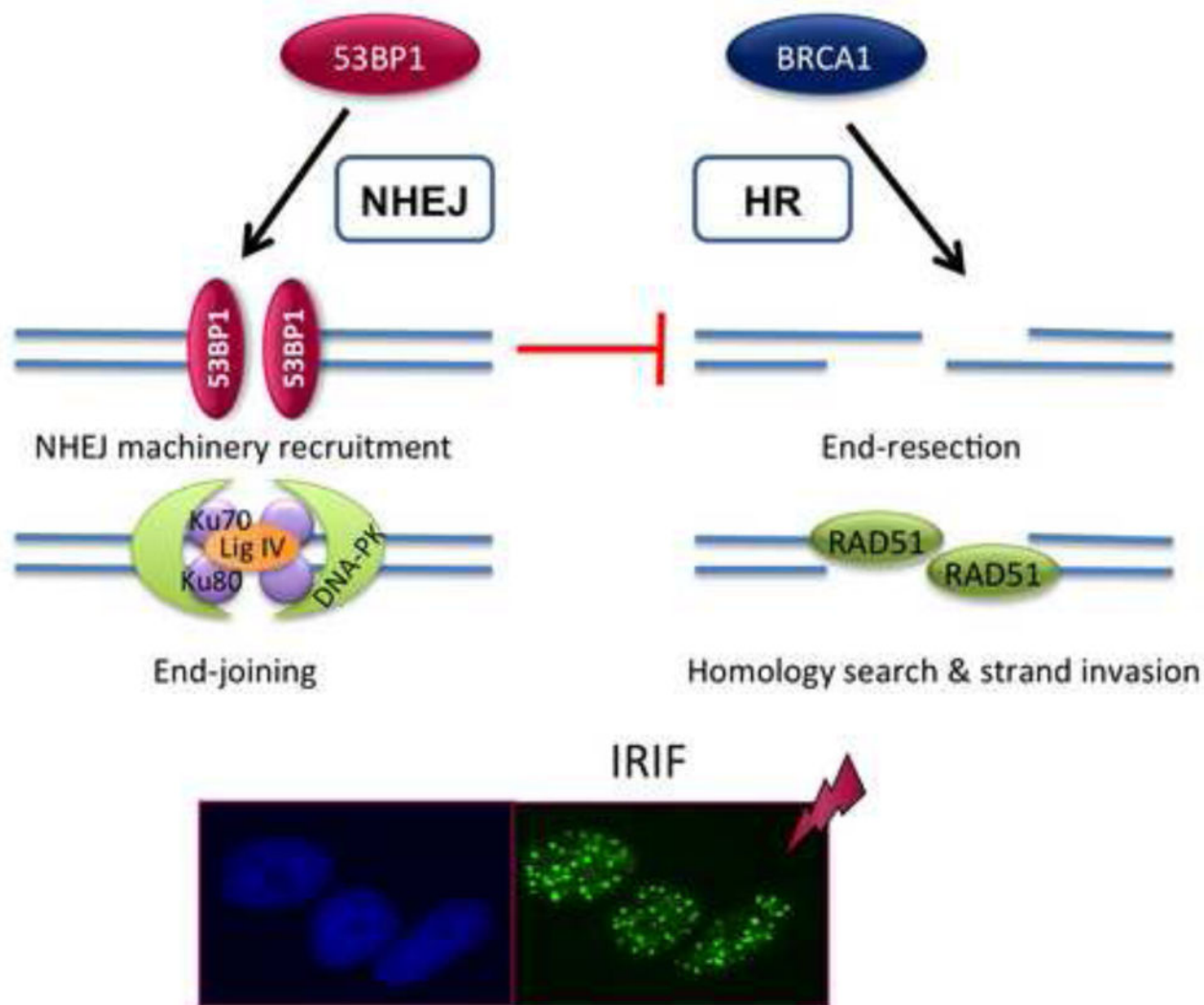
Figure 3. Relationship between 53BP1 and BRCA1 in DNA repair
53BP1 and BRCA1 proteins that have been recently in the spotlight for their key role
regulating the choice of mechanism of DNA DSB repair. BRCA1 protein promotes DNA
end-resection at the break, a prerequisite for binding of RAD51, a protein that is essential for
HR. In contrast, 53BP1 binding to the break facilitates the recruitment of the NHEJ
machinery. In addition, binding of 53BP1 prevents BRCA1-dependent end-resection and
thus functions as an inhibitor of HR. The recruitment of all these factors to DNA repair sites
can be easily visualized as foci by IF with specific antibodies. The image shows an example
of irradiated cells showing IRIF, and labeled with 53BP1 antibody.