Part-4

Cell cycle regulatory protein 5 (Cdk5)

A novel target of ERK in Carb induced cell death
1. Introduction

The process of replicating DNA and dividing cells can be described as a series of coordinated events that compose a cell cycle division (Kathleen et. al., 1997). Cell cycle plays a central role in normal growth and development (Annie and Roy 2003; Berthet and Kaldis 2007; Kiyokawa, 2006; Olashaw and Pledger 2002). Deregulation of cell cycle control leads to uncontrolled cell division, which is one of the initial event in the development of all types of cancers. At least two types of cell cycle control mechanisms are known; a cascade of protein phosphorylation that transmits a cell from one stage to the next and a set of checkpoints that monitor and control completions of critical events. The first type of control involves a highly regulated kinase family. Kinase activation generally requires association with second subunit that is transiently expressed at an appropriate period of the cell cycle; the periodic cyclin subunit associates with its partner “cyclin dependent kinase” (CDK) to create an active complex with unique substrate specificity (Kathleen et. al., 1997). Cyclin dependent kinases (Cdks) are the one, which control cell cycle and their discovery has always fascinated the cell biologist involved in drug-discovery. These kinases are positively regulated by cyclins (A, B, D and E) and are negatively regulated by cyclin dependent kinase inhibitors (CdkIs) (Sherr and Roberts 1999). The difference in pattern of cyclins expression and their specificity decides the relative position of a cell in the cell cycle. Due to overall importance of Cdks in cell cycle regulation, small molecules, which work as Cdk inhibitors, are widely in use for preclinical and clinical phases of cancer treatment (Olashaw and Pledger 2002; Sherr and Roberts 1999).
Neuron-specific cyclin dependent kinase 5 (Cdk5) is a unique member of small serine/threonine kinases family and is ubiquitously distributed in most tissues, including proliferating cells (Lin et. al., 2004). Cdk5 is primarily functional in post mitotic neuronal tissues. These cells specifically express the non-cyclin Cdk5 activator proteins, p35, p39 and a proteolytic fragment of p35 i.e. p25 (Lin et. al., 2004; Strock et. al., 2006; Tsai et. al., 1994). Interestingly, this neuron-specific Cdk5 was originally identified and cloned from cervical cancer HeLa cells (Maccioni et. al., 2001; Meyerson et. al., 1992). Till now, little is known about its functional role in cell cycle regulation (Meyerson et. al., 1992) or in cancer, though recently the expression and activity of Cdk5 has been reported in prostate (Lin, et. al., 2004; Strock et. al., 2006), breast (Goodyear and Sharma 2007) and medullary thyroid carcinoma cells (Lin et. al., 2007). All these studies collectively indicate that in addition to the role of Cdk5/p35 in central nervous system, it seems to have some extra-neural function(s), particularly, in cancer cells.

Cure for breast cancer is mainly dependent on combination of early detection and improved treatment. Chemotherapy is considered as the standard care in patient with primary breast cancer. Understanding the molecular mechanisms, particularly signaling pathways by which chemotherapeutic drugs work provides necessary information for an improved outcome. A number of promising novel targets of chemotherapeutic drugs are being evaluated in clinical trials to enhance their efficacy as well as therapeutic index. Present study is an attempt to investigate the involvement of Cdk5 in the proliferation as well as chemosensitivity of breast cancer cells. Though some preliminary reports in MCF-7 cells have indicated the presence of p35 protein (Xie et. al., 2003), Cdk5 expression at gene level (Laganiere et. al., 2005) and its activity (Song et. al., 2004), results presented here provide clear evidences that Cdk5 protein is not only expressed but is also active in MCF-7 cells. Additionally, consistent with these results,
here we demonstrate that Cdk5 positively regulates the proliferation of MCF-7 and MDA MB-231 cells. In addition, our data provides strong evidences for hyper activation of Cdk5 after Carb treatment. Activated Cdk5 was found to be involved in death of MCF-7 and MDA MB-231 cells. Carb treatment phosphorylates ERK which induces cell death in different cancer cell-types (Cagnol et. al., 2006; Lee and Kim 2007; Schweyer et. al., 2004; Singh et. al., 2007; Tang et. al., 2002; Wang et. al., 2000). In the present report we propose a novel mechanism of ERK mediated cell death in MCF-7 and MDA MB-231 cells following Carb treatment. Activation of Cdk5, a proposed downstream target of ERK, was regulated in ERK dependent manner. Moreover, we demonstrate that in wild type p53 expressing MCF-7 cells, p53 is one of the downstream effectors for Carb induced Cdk5 activation responsible for the induction of cell death. Overall, the data presented here not only has important implications in understanding of the molecular mechanisms underlying Carb induced cell death in breast cancer cells but also emphasizes the significance of Cdk5 activation in cancer cell death. To the best of our knowledge, this is the first evidence for implication of Cdk5 in conventional chemotherapeutic drugs induced cell death in any cancer cell-system.

2. Results and Discussion

2.1 MCF-7 and MDA MB-231 cells express active Cdk5 and p35 protein

In consistence with an earlier report, which mentioned that MCF-7 cells harbor Cdk5 activity (Song et. al., 2004), here, we provide experimental evidences to ascertain its presence as an active protein in these cells. In MDA MB-231 cells the expression and activity of Cdk5 has been reported recently (Goodyear and Sharma 2007). Likewise, the immunoblots performed from whole cell lysates of both
the cells using specific Cdk5 antibody also demonstrate that MCF-7 and MDA MB-231 cells express Cdk5 protein (Fig. 1A). Unlike other cyclin dependent kinases, cyclins do not regulate the activity of Cdk5. Catalytic activity of Cdk5 depends on its binding to p35, which is a well known activator of Cdk5 protein (Lew et. al., 1994; Lin et. al., 2004; Strock et. al., 2006; Tsai et. al., 2004).

Therefore, we next explored whether MCF-7 and MDA MB-231 cells also express p35 protein. To confirm that, western blot analysis of cell-lysates was performed by probing with specific p35 antibody. Our results indicate that p35 is expressed in both MCF-7 and MDA MB-231 cells (Fig. 1B). The presence of regulatory protein p35 led us to hypothesize that Cdk5 protein is indeed active in these cells. Therefore, to determine Cdk5 activity in these cells, kinase assays of immunoprecipitated Cdk5 from MCF-7 and MDA MB-231 cells lysates, prostate cancer cells DU-145 lysate was used as a positive control; (D) and (E) RS (20 µM) treatment for 36 h, inhibits Cdk5 activity in MCF-7 and MDA MB-231 cells respectively.

Fig. 1: Cdk5, p35 protein expression and Cdk5 activity in breast cancer cells. (A) western blot for Cdk5; (B) p35 in MCF-7 and MDA MB-231 cells; (C) kinase assays of immunoprecipitated Cdk5 from MCF-7 and MDA MB-231 cells lysates, prostate cancer cells DU-145 lysate was used as a positive control; (D) and (E) RS (20 µM) treatment for 36 h, inhibits Cdk5 activity in MCF-7 and MDA MB-231 cells respectively.
activity, *in vitro* kinase assay was performed in MCF-7 and MDA MB-231 cells. Prostate cancer cell line DU-145 (Lin et al., 2004; Strock et al., 2006) in Fig. 1C and roscovitine (RS) treated cells (Goodyear and Sharma 2007; Meijer et al., 1997) were used as a positive and negative control respectively, in kinase assay (Fig. 1 D, E). Our data suggest that in addition to earlier reports in DU-145 (Lin et al., 2004; Strock et al., 2006) and MDA MB-231 cells, Cdk5 is active in MCF-7 cells also (Fig. 1C, D). RS, a selective inhibitor of Cdk5 (Goodyear and Sharma 2007; Meijer et al., 1997) inhibits Cdk5 activity in MCF-7 and MDA MB-231 cells (Fig. 1 D, E).

2.2 Cdk5 protein is involved in breast cancer cell proliferation

To investigate the extra-neural function as well as physiological role of Cdk5 in MCF-7 and MDA MB-231 cells, we used two specific inhibitors, RS and Cdk2/5, in present study. These inhibitors block Cdk5 activity by competing for ATP binding domain of this kinase. To investigate the possible role of Cdk5 in cell proliferation, both these cells were treated with RS and Cdk2/5 for 36 h in dose dependent manner. The results clearly indicate that RS (Fig. 2A, B) or Cdk2/5 (Fig. 2C, D) treatment significantly reduced the survival of MCF-7 and MDA MB-231 cells in a dose dependent manner (Fig. 2). Interestingly, MCF-7 cells were more sensitive towards RS mediated cell growth inhibition in comparison to MDA MB-231 cells. Surprisingly the effect of Cdk2/5 inhibitor was almost similar in MCF-7 and MDA MB-231 cells (Fig. 2C, D). Our results clearly indicate that functionally active p53 expressing MCF-7 cells were more sensitive toward Cdk5 inhibitor, RS as compared to mutant p53 expressing MDA MB-231 cells (Fig. 2). These findings are in agreement with the reports proposing p53 involvement in induction of apoptosis in MCF-7 cells following RS.
treatment (Wesierska-Gadek et. al., 2005). Taken together, present results implicate Cdk5 involvement in breast cancer cell proliferation.

![Fig. 2: Cdk5 is involved in proliferation of breast cancer cells.](image)

**Fig. 2: Cdk5 is involved in proliferation of breast cancer cells.** Dose dependent effect of RS (A and B), and Cdk2/5 (C and D), on MCF-7 and MDA MB-231 cell-viability. MCF-7 and MDA MB-231 cells were exposed to increased concentrations of RS for 36 h in 96 well plates and cell viability was measured by MTT assay as described under material and method section. Absorbance given by untreated cells was taken as 100% cell survival. Results represent the mean ± SD of at least three independent experiments.

2.3 Cdk5 and its regulatory protein p35 expression increases after Carb treatment in MCF-7 and MDA MB-231 cells

In conditions such as ischemia, oxidative damage or other neurotoxic insults prolonged activation of Cdk5 result in the induction of apoptosis (Cheung et. al., 2004; Guo et. al., 2006; Maccioni et. al., 2001). Moreover, a recent report has also documented the Cdk5 dependent apoptosis in response to a chemotherapeutic drug Mitomycin-C in neuronal cells and suggested a growth inhibitory role of Cdk5 in response to stress (Lee and Kim 2007). Thus, it was of interest to explore whether Cdk5 activation was involved in Carb induced...
cytotoxicity in breast cancer cells. To examine the possible effect of Carb treatment on Cdk5 and p35 expression, MCF-7 and MDA MB-231 cells were treated with indicated doses of Carb for 36 h and assessed by immunoblot analysis. As shown in Fig. 3, Carb treatment resulted in increased Cdk5 protein expression in MCF-7 (Fig. 3A) and MDA MB-231 (Fig. 3B) cells. Simultaneously we also observed an increase in p35 protein expression in both the cells following Carb treatment (Fig. 3A, B).
Fig. 3: Expression of p35, Cdk5 and activity of Cdk5 increases following treatment with Carb. MCF-7 (A) and MDA MB-231 (B) cells were treated with increasing concentration of Carb for 36 h. In all the panels, cells were harvested and equal amount of protein was processed for western blot analysis, to detect p35 and Cdk5 protein expression. Membranes were stripped and reprobed for β-Actin to ensure equal protein loading. These results are representative of three independent experiments. Densities of the bands were quantified by image analyzer and fold expressions are with reference to control cells. In kinase assay (C and D), cell lysates were subjected to Cdk5 immunoprecipitation followed by anti-Cdk5 immunoblotting and in vitro (32P) ATP kinase assay using Histone H1 as the substrate. Densities of the bands were quantified by image analyzer. Results displayed in the bar graphs are quantitative analytic data from three independent experiments and presented as the mean ± SD.
2.4 Carb treatment increases the Cdk5 activity in MCF-7 and MDA MB-231 cells

Next, we investigated the changes in Cdk5 activity following Carb treatment by *in-vitro* Cdk5 kinase assay in MCF-7 and MDA MB-231 cell-lysates (treated as well as untreated cell lysates were incubated with Histone H1 as described in material and method section. We observed almost 1.6 fold increase in Cdk5 activity (as indicated by increased phosphorylation of Histone H1) in Carb treated cells (Fig. 3C, D). In all kinase assays, RS treated cells were used as negative control to ascertain specificity. Collectively, our data clearly suggests that Carb not only increases Cdk5/p35 protein expression but also enhances Cdk5 activity in both these cells. An increase in p35 and Cdk5 expression has been recently documented in DNA damage induced apoptosis of neuronal cells (Lee and Kim 2007). This data suggests that Cdk5 may mediate a novel pathway of Carb induced cell death in MCF-7 and MDA MB-231 cells.

2.5 Inhibition of Cdk5 activity promotes cell survival in Carb treated cells

To explore the possible involvement of Carb induced Cdk5 activity in chemosensitivity, MCF-7 and MDA MB-231 cells were treated with Carb in the presence or absence of Cdk5 specific inhibitor and cell viability was assessed by MTT assay. Cells were pretreated with indicated doses of RS or Cdk2/5 inhibitor one hour prior to the treatment with Carb. As shown in Fig. 4 A, B, when either of the cell lines was treated with IC_{50} dose of Carb, approximately 50% cell death was observed. However, when MCF-7 cells were treated with IC_{50} dose of Carb in the presence of increasing dosage of Cdk2/5 inhibitor, approximately 70% cell survived (Fig. 4A).
A similar trend of increase in cell survival was also observed in MDA MB-231 cells treated with Carb in presence of either RS or Cdk2/5 in comparison to Carb alone treated cells. Surprisingly, RS pretreatment resulted in increased cell survival only in MDA MB-231 cells (Fig. 4B). Whereas, in RS pretreated MCF-7 cells, an increase in cell death after Carb treatment in comparison to drug alone treated cells was observed (Fig. 4C). This difference may be due to some specific molecular effects of RS on MCF-7 cells (Maggiorella et. al., 2005; Mgbonyebi et. al., 1998; Wesierska-Gadek et. al., 2005; Wesierska-Gadek 2006a). RS induced cell death in MDA MB-231 cells also, which seems to be
p53 independent. Collectively, for the first time these results not only directly implicate Cdk5 activation in Carb triggered death of MCF-7 and MDA MB-231 cells but also provide clear evidences of dual function of Cdk5, a mediator of cell survival or cell death as in case of neuronal-system (Cheung, 2004; Guo, 2006).

2.6 Carb activated ERK induced cell death in MCF-7 and MDA MB-231 cells

DNA damage induced by chemotherapeutic drugs like mitomycin C, etoposide (Tang et. al., 2002), cisplatin (Schweyer et. al., 2004; Wang et. al., 2000), carboplatin (Singh et. al., 2007) or by UV irradiation (Coffer et. al., 1995; Guo 2006) activate ERK, which plays a central role in the induction of apoptosis in various cell-types. In parallel with these reports, we observed that in addition to Cdk5 activation, ERK is also activated following Carb treatment in MCF-7 and MDA MB-231 cells (Fig. 5). As shown in Fig. 5A, B, Carb treatment activated ERK in a time dependent manner, in MCF-7 and MDA MB-231 cells. Further, to define the functional involvement of ERK activation in Carb induced cell death both the cells were pretreated with 20 \( \mu \)M of U0126 (a selective inhibitor of ERK), one hour prior to Carb treatment. As shown in Fig. 5C, in presence of ERK inhibitor approximately 70-75% cells survived compared to survival of 50% cells in Carb alone treated cells. Also, the survival of both the cells was inhibited by U0126 in a dose dependent manner, which suggests that basal ERK activity is essential for cells survival. Collectively, these results indicate that fundamentally basal ERK and Cdk5 activities play a role in survival of MCF-7 and MDA MB-231 cells (Fig. 5 D, E) whereas, stress (like Carb induced DNA damage) enhanced activation of ERK or Cdk5, promotes cell death.
These results ascertained that it is indeed the activated ERK, which is involved in Carb induced cell death. Many earlier reports in different cell-system also support present finding and strengthen the impact of present study indicating the central involvement of ERK activation in cell death (Cagnol et. al., 2006; Lee and Kim 2007; Schweyer et. al., 2004; Singh et. al., 2007; Tang et. al., 2002; Wang et. al., 2000).
2.7 Carb induced ERK utilizes Cdk5 as one of its downstream target

Many conflicting reports in neuronal cells suggest that ERK activation may be either upstream (Lee and Kim 2007) or downstream of Cdk5 (Sharma et. al., 2007; Zheng et. al., 2007), which probably depends on the normal or stressed condition of the cells. Thus, we next investigated whether there is a direct link between ERK and Cdk5 (Lee and Kim 2007; Sharma et. al., 2007; Zheng et. al., 2007) in breast cancer MCF-7 and MDA MB-231 cells. Inhibition of ERK activity by U0126 significantly blocked Cdk5 kinase activity in MCF-7 and MDA MB-231 cells (Fig. 6A, B). Importantly, treatment with ERK inhibitor not only decreased basal Cdk5 activity, but also diminished Carb induced
Cdk5 activation. This result provides a strong evidence for ERK being an upstream activator of Cdk5 in MCF-7 and MDA MB-231 cells. Collectively, our data strongly suggest that ERK and Cdk5 kinase coordinate at basal level which is important in cell survival but at the same time in response to Carb treatment their activation results in cell death in MCF-7 and MDA MB-231 cells.

**Fig. 6:** Blocking of basal as well as Carb induced ERK activation inactivates Cdk5 in cells. MCF-7 (A) and MDA MB-231 (B) cells were treated with indicated concentration of U0126 (20 µM), Carb (450 µM in MCF-7; 400 µM in MDA MB-231) or RS (20 µM) as per experimental requirement. In all panel inhibitor(s) or drug treatment were given for 36 h either alone or in combination. Cells were treated with U0126 1 h prior to addition of Carb and further exposed to inhibitor for 36 h. Cdk5 kinase activity was examined by autoradiography using Histone H1 as a substrate, as given in material and method section. Densities of the bands were quantified by image analyzer. Results displayed in the bar graphs are quantitative analytic data from three independent experiments and presented as the mean ± SD.
2.8 p53 protein expression and transactivation activity increased after Carb treatment

p53 is one of the major effectors in the DNA damage response and various reports have suggested that Cdk5 regulates p53 activity by its phosphorylation in neuronal cells (Lee et. al., 2007; Wesierska-Gadek et. al., 2006b). We explored the possibility of a link between p53 and Cdk5 in a wild type p53 expressing MCF-7 cells. Therefore, we examined the effect of Carb treatment on p53 expression and activity in MCF-7 cells. As shown in Fig. 7A, p53 protein levels increased in a time dependent manner after Carb treatment. Further, cells were co-transfected with a reporter construct containing p53 response element (pG13CAT) along with a control pEGFP plasmid. p53 promoter is significantly activated in cells treated with Carb as compared to untreated cells (Fig. 7B).
2.9 p53 is a down-stream target of Cdk5 in MCF-7 cells

It has been reported that Cdk5 regulates p53 phosphorylation as well as its activation in neuronal cells (Lee and Kim 2007; Lee et. al., 2007; Wesierska-Gadek et. al., 2006b). To investigate whether Cdk5 mediates cell death in MCF-7 by regulating activity of p53, MCF-7 cells were transfected with pG13CAT expression vector and subsequently
treated with indicated dosage of Carb in presence or absence of Cdk5 inhibitor as described in material and method. Interestingly, we observed that both RS and Cdk2/5 inhibitor markedly inhibited Carb induced p53 promoter activation (Fig. 8A). To validate these results we further checked p53 regulated p21 gene promoter activation in presence of Cdk5 inhibitor(s). As shown in Fig. 8B, p21 promoter CAT reporter activity was also inhibited in Carb treated MCF-7 cells in the presence of RS and Cdk2/5. Interestingly both RS and Cdk2/5 alone activate p53 or p21 promoter which also supports the fact that Cdk5 is involved in the proliferation of these cells via p53. Here it is important to note that RS induced p53 or p21 promoter activation in significantly more than that induced by Cdk2/5. Cdk5 mediated cell death in MDA MB-231 cells is functionally independent of p53 activation as p53 is mutated in these cells. Our data provide a basis to state that Cdk5 activation involved in cell death induced by Carb is likely to be independent of p53 status. However, in the cells in which p53 is functional, activated Cdk5 utilizes p53 as one of its downstream target for the induction of cell death.
Fig. 8: Carb induced p53 and p21 promoter activation is blocked by Cdk5 inhibitor. Carb induced p53 transactivation activity was suppressed by the treatment of RS and Cdk2/5 and demonstrated by CAT assay (A and B). MCF-7 cells were treated with 450 µM of Carb for 36 h with or without indicated concentration of RS (20 µM) or Cdk2/5 (25 µM) inhibitor(s). In all inhibitor and drug combination panel, inhibitor treatment was given 1 h prior to addition to Carb and further exposed in presence of inhibitor for 36 h. CAT assays were performed as per protocol described in material and method. Densities of the bands were quantified by image analyzer. Results displayed in the bar graphs are quantitative analytic data from three independent experiments and presented as the mean ± SD.
3. Conclusions

In the way of finding novel targets of a chemotherapeutic drug, present study is based on exception from the rule that Cdk5 activity is present only in the brain cells though there are some evidences suggesting Cdk5 involvement in the proliferation of a number of non-neuronal cell-types also (Goodyear and Sharma 2007; Lin et. al., 2004; Lin et., al., 2007; Strock et., al., 2005). Some of these upcoming reports not only document the presence of Cdk5 activity in cancer cells but also implicate its functionality in various important aspects of cancer cells including cellular proliferation (Goodyear and Sharma 2007; Lin et. al., 2007), metastasis (Strock et. al., 2006) and apoptosis (Lin et. al., 2004). Moreover, a recent report has also documented that chemotherapeutic drug Mitomycin-C induced apoptosis in neuronal cells and suggested a growth inhibitory role of Cdk5 in response to stress (Lee and Kim 2007). Thus, it was of interest to explore whether the Cdk5 activation was involved in Carb induced cytotoxicity in breast cancer cells. Our data clearly suggest that Carb treatment not only increases the expression of Cdk5 and its activator protein, p35 but also enhances Cdk5 activity (Fig. 3A, B, C, D). We also investigated whether Carb induced enhancement in Cdk5 activity is involved in cell death in MCF-7 and MDA MB-231 cells. Both MCF-7 and MDA MB-231 cells, in absence or presence of RS and or Cdk2/5, were treated with Carb for 36 h. Interestingly, it was observed that inhibition of drug induced Cdk5 activation enhanced the survival of cells as compared to Carb treatment alone (Fig. 4 A, B). These results indicated that Carb induced cell death is in-part dependent on the increased Cdk5 activity in MCF-7 and MDA MB-231 cells. Various groups have assigned a central role to ERK activation in the induction of apoptosis by different agents. Very interestingly, in parallel with the ERK activation our
present finding also demonstrates the involvement of Cdk5 activity in the proliferation as well as in death of MCF-7 and MDA MB-231 cells following Carb treatment. Therefore, we explored whether activation of ERK is involved in Carb induced cell death in MCF-7 and MDA MB-231 cells. Carb treatment in MCF-7 and MDA MB-231 cells led to sustained ERK activation in a dose dependent manner (Fig. 5A, B). To investigate the role of Carb induced ERK activation in cell death; both the cells were treated with Carb in presence or absence of ERK inhibitor U0126. In U0126 pretreated MCF-7 and MDA MB-231 cells, survival increased significantly in comparison to drug alone treated cells (Fig. 5C). This provides strong evidence that Carb induced ERK activation plays an important role in cell death. These results ascertained that it is indeed the activated ERK, which is involved in Carb induced cell death. Many earlier reports in different cell-system also support present finding and strengthen the impact of present study indicating the central involvement of ERK activation in cell death (Cagnol et. al., 2006; Lee and Kim 2007; Schweyer et. al., 2004; Singh et. al., 2007; Tang et. al., 2002; Wang et. al., 2000). As documented in neuronal system (Lee and Kim 2007; Sharma et. al., 2007; Zheng et. al., 2007) and as well supported by our data in MCF-7 and MDA MB-231 cells, the basal activity of ERK or Cdk5 promotes cell proliferation and their enhanced activation is involved in cell death. This functional similarity in ERK and Cdk5 suggests a possible link between these two important signaling molecules. Based on independent results of Cdk5 kinase assays, we have demonstrated for the first time that U0126, the inhibitor of MEK/ERK, significantly decreased basal as well as Carb induced Cdk5 activation in MCF-7 and MDA MB-231 cells (Fig. 6A, B). p53 is one of the major effectors in the DNA damage response and various reports have suggested that Cdk5 regulates p53 activity by its phosphorylation in neuronal cell (Lee et. al., 2007; Wesierska-Gadek et. al., 2006b). Thus, we reasoned the possibility of a link between p53
and Cdk5 in wild type p53 expressing MCF-7 cells. Importantly, we observed that not only p53 protein levels were elevated but also p53 promoter was activated in response to Carb treatment (Fig. 7). These results implicate p53 as a downstream transcriptional factor target of Cdk5 in Carb treated MCF-7 cells. As shown in Fig. 8, not only a significant decrease in promoter activity of p53 was detected but also decreased promoter activation of one of its downstream targets, p21 was observed following Carb treatment in presence of Cdk5 inhibitors as compared to Carb alone treated cells. Taken together, our data provide a strong basis to state that MCF-7 and MDA MB-231 cells express functional Cdk5 protein. The basal activity of Cdk5 is essential for proliferation of these cells. We propose a novel mechanism of ERK induced cell death involving Cdk5 as a molecular target of ERK in Carb treated MCF-7 and MDA MB-231 cells. Moreover, ERK activation is associated with cell death in both the cells via Cdk5 activation, independent of p53 status. These results suggest that the Cdk5 mediated downstream apoptotic pathway may be p53 dependent as well as p53 independent (Fig. 9). Overall, this report for the first time assigns a key role to Cdk5 as one of the regulators in Carb induced cell death and suggests that Cdk5 can be potentially used as a novel target of chemotherapeutic drug for better treatment of breast cancer. The propose model is depicted in Fig. 9.
Fig. 9: Cell cycle regulatory protein 5 (Cdk5) is a novel target of Carb. DNA damage stress enhanced ERK activity utilizes Cdk5 as one of its downstream targets for the execution of death signal in Carb induced death in MCF-7 and MDA MB-231 cells. Activated Cdk5 modulates p53 promoter activation in MCF-7 cells. However, in p53 mutant MDA MB-231 cells, Cdk5 mediated cell death may be p53 independent.