We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

11

Molecular Targets of Benzyl Isothiocyanates in Pancreatic Cancer

Srinivas Reddy Boreddy, Kartick C. Pramanik and Sanjay K. Srivastava
Department of Biomedical Sciences
Texas Tech University Health Sciences Center, Amarillo, TX
USA

1. Introduction

Addiction of cancer cells to survival pathways has been well documented in most of the cancer models including the pancreatic cancer. Pancreatic cancer is one of the most aggressive tumors with an average five year survival rate of less than 5% (Jemal A et al., 2010). It is associated with high expression levels of various survival pathways, such as KRAS, STAT3, AKT, NFkB, HDAC etc. Furthermore, pancreatic cancer acquires resistance to various apoptosis signals such as FasL, TRAIL. In addition, pancreatic cancer gets resistance to various chemo-drugs including gemcitibine by altering survival pathways.

Currently, there is no effective treatment for pancreatic cancer because conventional chemotherapy including the gemcitabine and 5-FU, and radiation treatment has shown very limited success in improving the patient survival. Therefore, the development of novel approaches to prevent and treat pancreatic cancer is an important mission.

Evidence from epidemiological, pharmacological, and case-control studies continue to support the notion that isothiocyanates (ITCs) present in cruciferous vegetables may have substantial chemopreventive activity against various human malignancies including pancreatic cancer (Zhang Y et al., 1992); Stoner GD & Morse MA, 1997). Benzyl isothiocyanate (BITC), an agent that is present in cruciferous vegetables such as, watercress, cabbage, cauliflower, mustard, and horseradish, is widely consumed as part of a routine diet. BITC has been reported to inhibit initiation, growth, and metastasis of human cancers in rodents (Batra S et al., 2010; Boreddy SR et al., 2011a; Boreddy SR etal., 2011b; Kim EJ et al., 2011; Sahu RP & Srivastava SK, 2009; Zhang Y et al., 1992). The structure of BITC is shown in Fig.1.

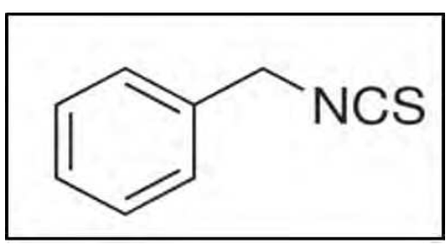


Fig. 1. Chemical structure of BITC

Our laboratory has shown that BITC potentially suppresses the growth and induces apoptosis in pancreatic cancer cells by targeting various key survival molecules (Batra S et al., 2010; Boreddy SR et al., 2011a; Sahu RP & Srivastava SK, 2009). In the present chapter, we discuss the current understanding of BITC, its targets and mechanism of action in pancreatic cancer.

2. Effect of BITC on STAT3 signalling pathway

Signal Transducer Activator Transcription (STAT3) transcription factors are latent proteins that bind to the genome on activation to either induce or to repress gene expression (Bromberg et al., 1999). STAT3 is aberrantly activated in majority of the cancers including pancreatic cancer (Wei et al., 2003). Clinical specimens have revealed that more than 50% of the breast and lung cancer, and over 95% of head and neck cancers have hyperactive STAT3 signaling (Darnell, 2005). Interestingly, STAT3 deficient mice in a chemical carcinogenesis model have shown the reduced proliferation of epithelial cells due to inability to pass through G1-S-G2 cell cycle progression (Chan et al., 2004). Furthermore, Chiarle et al. have demonstrated that disruption of STAT3 signaling by anti-sense oligoneclosides was sufficient to impair the growth of solid tumors (Chiarle, 2005), highlighting the potential of anti-STAT3 therapy in clinical medicine. Recently, numerous natural and synthetic compounds have been discovered to target STAT3 signaling. Results from our laboratory showed that benzyl isothiocyanate (BITC) targets STAT3 signaling to induce apoptosis in pancreatic cancer (Sahu & Srivastava, 2009).

Our laboratory showed that BITC significantly suppress the phosphorylation of STAT3 at both Tyr-705 and Ser-727 to induce apoptosis in BxPC-3 (Fig. 2), MIA PaCa-2, Capan-1 and PanC-1 pancreatic cancer cell lines, in a dose and time dependent manner (Sahu & Srivastava, 2009). Interestingly, BITC also down regulated the protein levels of STAT3 in these cell lines, although its functional implications are yet to be explored. Furthermore, down regulation of STAT3 protein expression by BITC was transcriptional, as evidenced by RT-PCR analysis of BITC treated BxPC-3 cells (Fig. 2).

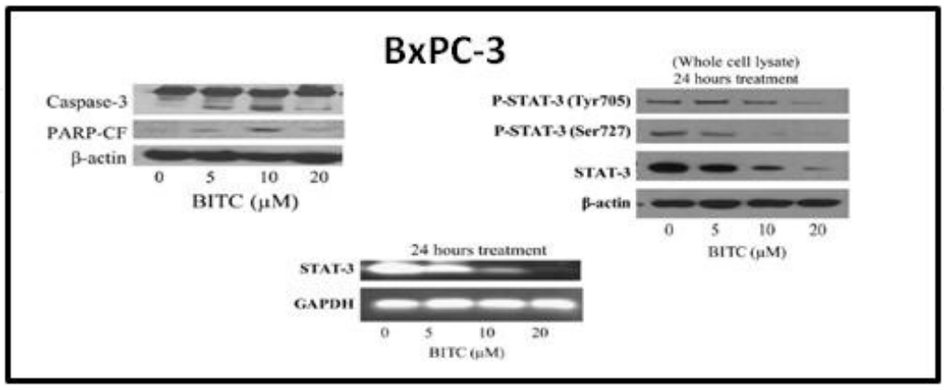


Fig. 2. Benzyl isothiocyanate induces apoptosis in pancreatic cancer cells by inhibiting the phosphorylation of STAT3. (J Natl Cancer Inst 2009;101: 176 – 193).

BITC-induced apoptosis was further substantiated by IL-6 treatment, which specifically phosphorylates STAT3 at Tyr-705 (Berishaj, 2007) and STAT3α overexpression. IL-6 pre-

treated BxPC-3 cells showed significant resistance to BITC-induced apoptosis (Fig. 3). Similarly, when STAT3α was over expressed in BxPC-3 cells, BITC-induced apoptosis was severely abrogated, indicating that BITC targets STAT3 to induce apoptosis in pancreatic cancer cells.

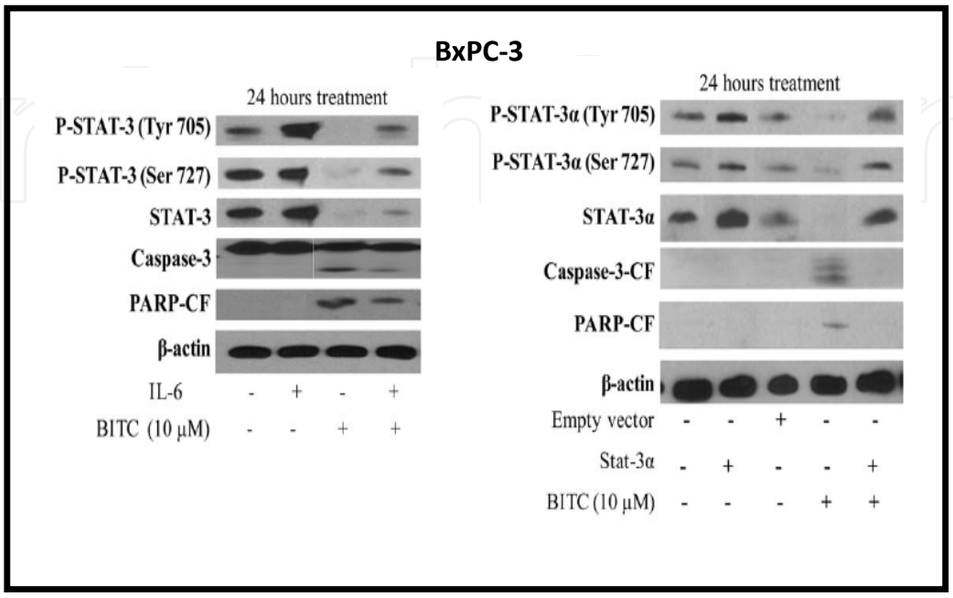


Fig. 3. IL-6 pre-treatment or STAT3α overexpression abrogates BITC-induced apoptosis in pancreatic cancer cells. (J Natl Cancer Inst 2009:101; 176 – 193).

3. Effect of BITC on AKT/FOXO/Bim signaling pathway

Phosphotidyl inositol 3phosphate (PI3K)/AKT signaling plays a critical role in cell survival and growth during embryonic development as well as during normal cell survival (Finkielsztein & Kelly, 2009). However, cancer cells exploit the same pathway to overcome apoptosis induced by either therapeutic drugs or internal stimuli such as oxidative stress. Upon binding of growth factors to Tyrosine Kinase Receptor (TKR), PI3K is directly or indirectly activated by TKRs by inhibiting or removing the P85 regulatory unit of PI3K (Vivanco & Sawyers, 2002). Activated PI3K phosphorylates phosphatidylinositol and converts inositol 4,5biphosphate (PIP2) into PIP3. Consequently, AKT and PDK translocate to membrane and interact with PIP3 through PH domain leading to conformational changes in AKT to expose phosphorylation sites. AKT is phosphorylated by PDK1 at Ser-308 leading to stabilization of AKT. Yet another phosphorylation takes place at Tyr-473, which is required for full activation of AKT. In addition, another protein complex mTOR has been shown to be required for the phosphorylation of AKT (Sarbassov et al., 2005). This pathway is negatively regulated by phosphatases, such as PTEN, which dephosphorylates PIP3 thus limiting its availability (Osaki et al., 2004).

Recently, FOXO transcription factors received ample of attention in cancer because of direct involvement in apoptosis and drug resistance (Salih & Brunet, 2008). FOXO1 and FOXO3a are the members of FOXO transcription factors, which operate right under the AKT signaling. Upon growth signal stimulation, AKT is activated by phosphorylation at Ser-473,

which further phosphorylates FOXO1 or FOXO3a transcription factors. Phosphorylated FOXOs bind to 14-3-3 chaperons and transported out of nucleus and subjected to proteosomal degradation (Tzivion et al., 2011). But during oxidative stress or growth factor withdrawal, AKT is dephosphorylated leading to nuclear import of FOXOs and induction of pro-apoptotic proteins such as Bim and PUMA (Obexer, 2011).

A recent report has shown that 59% of the pancreatic tumors harbor aberrantly activated AKT signaling (Schlieman et al., 2003). One of the possible reasons behind hyperactive AKT signaling in pancreatic cancer is due to mutation or deletion of PTEN gene (Sawai et al., 2008). Indeed, strategies aimed at blocking AKT activation could be a promising treatment for pancreatic cancer. Interestingly BITC significantly inhibited AKT signaling *in vitro* and *in vivo.*

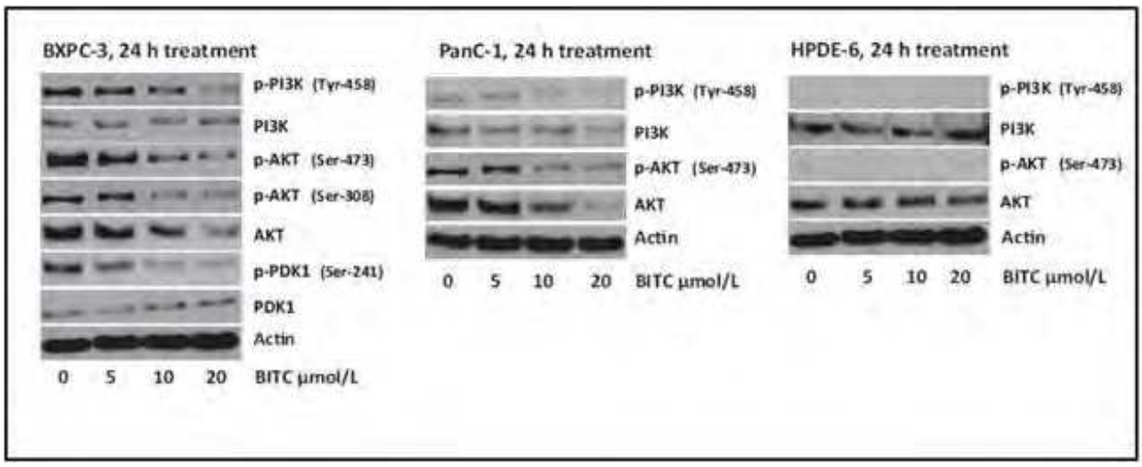


Fig. 4. BITC down regulates the phosphorylation of key molecules of PI3K/AKT pathway. (Clin Cancer Res; 17(7); 1784–1795).

BITC suppressed the phosphorylation of AKT at both Ser-308 and Ser-473 in BxPC-3 and PanC-1 cells, in dose dependent manner (Boreddy et al., 2011a). Furthermore, BITC also suppressed the phosphorylation of various other key molecules of PI3K/AKT pathway such as PI3K (Tyr-458), PDK1 (Ser-241), mTOR (Ser-2448) etc. (Fig. 4), indicating that BITC targets PI3K/AKT signaling to induce apoptosis in pancreatic cancer cells. Interestingly, BITC was almost ineffective in human pancreatic ductal epithelial (HPDE-6) cells (Fig. 4). Over expression of AKT blocked the apoptosis inducing effects of BITC in pancreatic cancer cells.

Recently, FOXO transcription factor received plenty of attention as a potential target for cancer therapy, as they are directly involved in apoptosis induction. Interestingly, BITC significantly suppressed the phosphorylation of FOXO1 (Ser-256) and FOXO3a (Ser-253), without effecting the protein levels in both BxPC-3 and PanC-1 cells (Fig. 5). Moreover, immunoprecipitation studies showed that BITC treatment significantly masked 14-3-3 binding motif on FOXO proteins indicating that more of FOXO proteins were retained in the nucleus (Fig. 5B). Furthermore, BITC significantly increased the expression FOXO1 transactive genes such as P21, P27 and Bim in both the cell lines, BxPC-3 and PanC-1 (Fig. 5C).

Apart from phosphorylation, another tier of FOXO transcription factor regulation is acetylation. Interestingly, BITC also reduced the acetylation of FOXO proteins. Probably, inhibition of acetylation by BITC was due to down regulation of CBP protein expression, since SIRTs were not altered by BITC treatment.

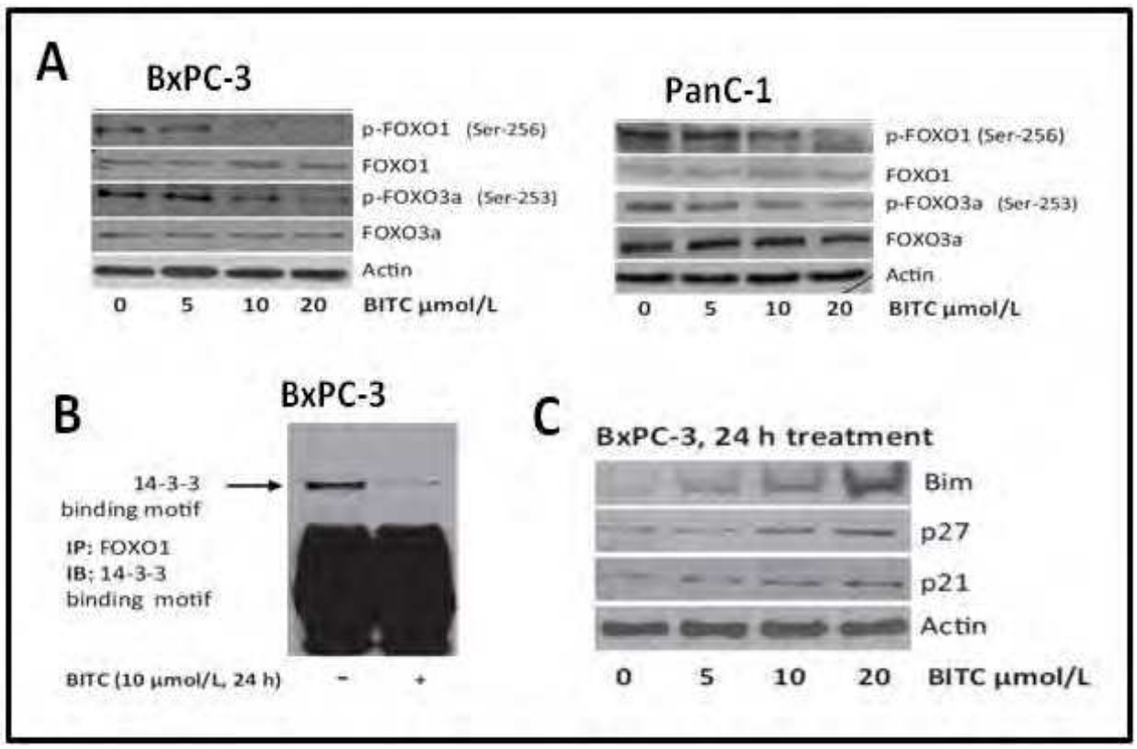


Fig. 5. BITC activates FOXO transcription factors and pro-apoptotic proteins in pancreatic cancer cells. (Clin Cancer Res; 17(7); 1784–1795).

Role of AKT in BITC-induced apoptosis was further confirmed by using PI3K inhibitor LY-294002 and overexpression of wild type AKT in BxPC-3 cells. Interestingly, when BxPC-3 cells were pre-treated with LY-294002 followed BITC (10µM) for 24h, apoptosis induction was potentiated, as compared to BITC alone treated cells (Fig. 6), whereas AKT overexpression severely abrogated BITC-induced apoptosis in BxPC-3 cells (Fig. 6). In line with apoptosis results, phosphorylation of FOXO proteins were increased with AKT overexpression, whereas Bim, P27, P21 expression was reduced. However, BITC partially blocked these effects, indicating that BITC targets AKT pathway to induce apoptosis in pancreatic cancer cells lines (Fig. 6).

4. BITC Regulates NFkB Activity by Inhibiting HDACs

NFkB transcription factors are mainly involved in the regulation of immune and inflammatory response, apart from cell proliferation and apoptosis Ghosh et al., 1998; Hart et al., 1998). NFkB is normally located in the cytoplasm sequestered by its endogenous inhibitor IkB. Upon cellular stimulation, IkB proteins are phosphorylated at Ser-32/36 liberating NFkB, which translocates to the nucleus and gets involved in the transcription of responsive genes such as Cyclin D1 (Sun & Andersson, 2002).

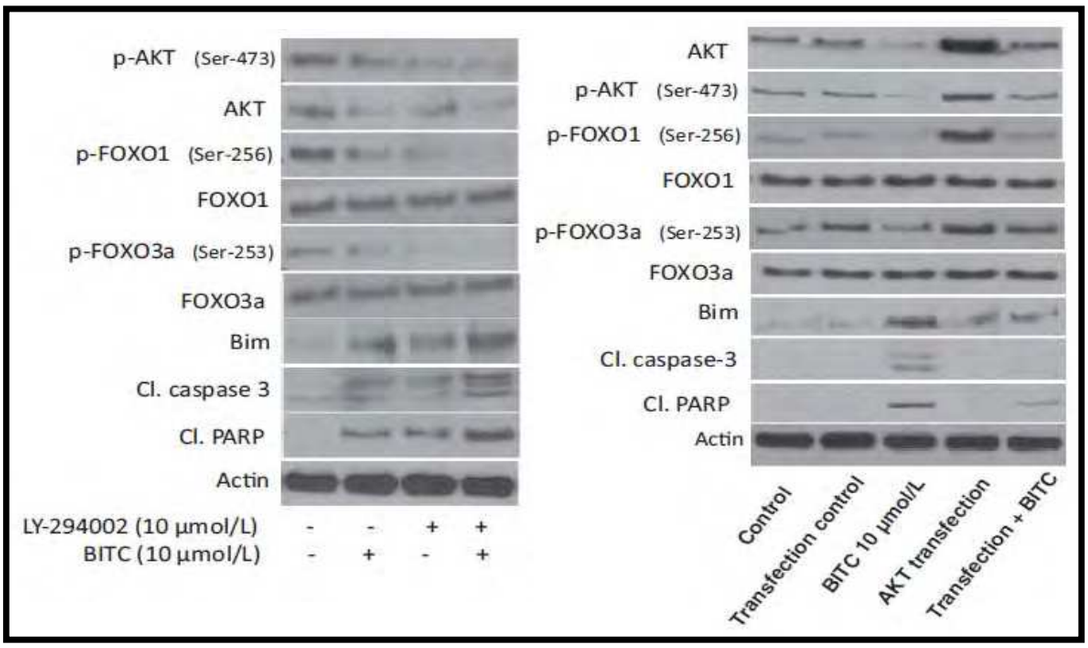


Fig. 6. AKT inhibitor potentiates BITC-induced apoptosis, whereas AKT overexpression abrogates BITC-induced apoptosis in BxPC-3 pancreatic cancer cells. (Clin Cancer Res2011: 17(7); 1784–1795).

NFkB may activate various survival signals to promote cell survival. NFkB is known to interfere with inducers of extrinsic apoptosis pathway by up regulating the FLIP-like inhibitory protein (Kreuz et al., 2001). NFkB also induces the expression of inhibitors of apoptosis proteins such as IAP (Deveraux et al., 1998) and some members of the Bcl2 (Shou et al., 2002) family proteins, thereby protecting the cells from various apoptosis stimuli. NFkB is also known to play critical role in drug resistance is various cancer models (Arlt et al., 2003). Hence, inhibiting of NFkB activation may potentiate the clinical efficacy of the drugs.

BITC significantly inhibits the phosphorylation of NFkB at both Ser-276 and Ser-536 in both BxPC-3 and Capan-2 pancreatic cancer cells, in a dose and time dependent manner (Fig. 7A&B). Interestingly, BITC down regulated the expression of NFkB in BxPC-3 cells but not in Capan-2 cells, indicating that BITC differentially act on different cells (Batra et al., 2010). Furthermore, BITC drastically inhibited the nuclear localization of NFkB in BxPC-3 cells (Fig. 7C). BxPC-3 cells that were transfected with a luciferase gene containing NFkB-promoter and treated with BITC demonstrated around 90% decrease in luciferase activity, as compared to control cells (Fig. 7D). Furthermore, BITC also decreased Cyclin D1 expression and transcriptional activity, as it is one of the target genes of NFkB (Fig. 7E & Fig. 7F). Interestingly expression of IKK was decreased with BITC treatment, but neither phosphorylation (Ser32/36) nor protein levels of IkB were altered in BITC treated BxPC-3 cells (Fig 7A), indicating that down regulation of IKK by BITC treatment could be the reason for inhibition of NFkB phosphorylation (Ser-536).

Apart from the phosphorylation, NFkB is known to be regulated by acetylation. Interestingly, BITC also inhibited the acetylation of NFkB on lysine residue in BxPC-3 cells. BITC suppressed the acetylation of NFkB by altering the expression of HDAC1 and HDAC3

(Fig. 8A&B), as these molecules play critical role in NFkB acetylation. In agreement with other HDAC inhibitors' data, such as veronistat (SAHA) and tricostatin A (TSA), BITC also up regulated the expression of p21 in BxPC-3 and Capan-2 cells, in a dose dependent manner (Fig. 8C).

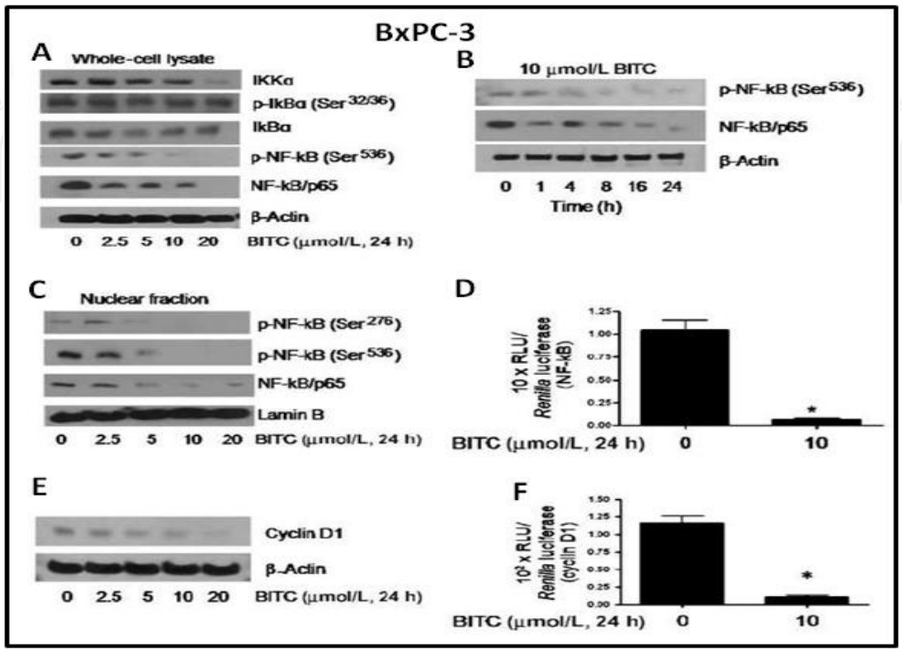


Fig. 7. BITC treatment causes inhibition of NF-κB and cyclin D1 in BxPC-3 pancreatic cancer cells. (Mol Cancer Ther 2010: 9(6):1596-608).

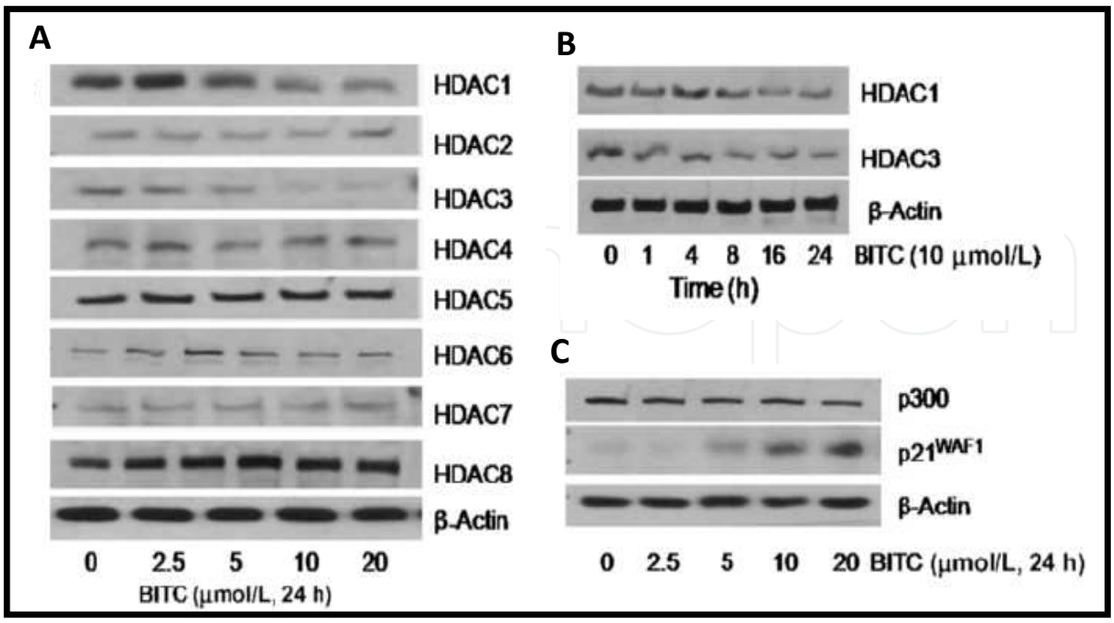


Fig. 8. BITC down regulates the expression of HDACs and p21 in BxPC-3 cells. (Mol Cancer Ther. 2010: 9(6);1596-608).

Role of HDACs in BITC-induced NFkB deacetylation was further substantiated by HDAC overexpression in BxPC-3 cells. HDAC1/3 overexpression significantly outweighed the effects of BITC in BxPC-3 cells. Furthermore, overexpression of HDACs protected BxPC-3 cells from BITC-induced apoptosis, as indicated by the reduced cleavage of caspase-3, PARP and increased survival in HDACs overexpressing BxPC-3 cells, as compared to BITC alone treated cells (Fig. 9).

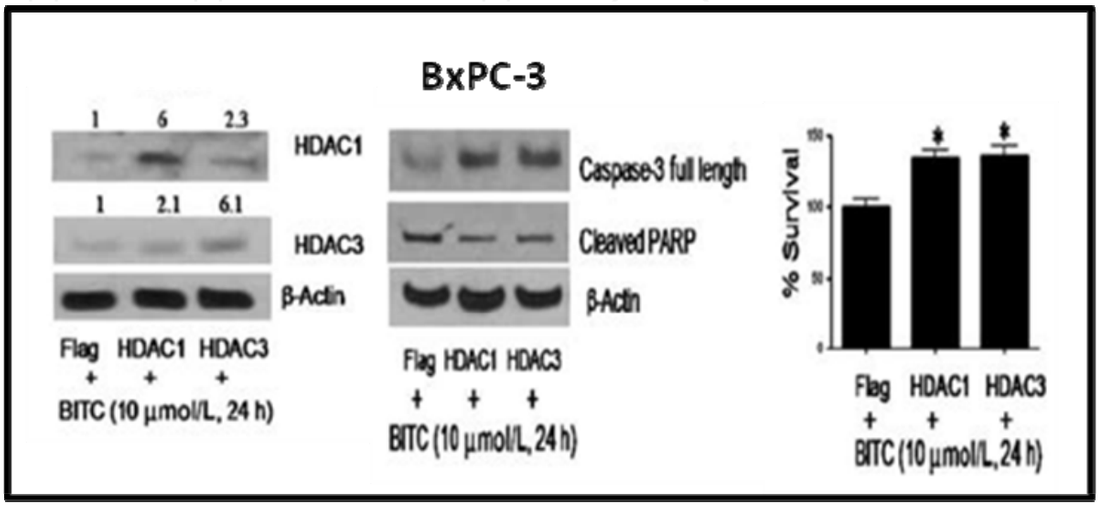


Fig. 9. Over expression of HDAC1/HDAC2 rescue BxPC-3 cells from BITC-induced apoptosis. (Mol Cancer Ther. 2010: 9(6);1596-608).

5. BITC induces ROS generation, DNA damage and cell cycle arrest in pancreatic cancer cells

As many drugs induce cell death in cancer cells by triggering ROS generation, it was quite obvious to see whether BITC could induce ROS generation in pancreatic cancer cells. In agreement with other drugs, BITC caused significant generation of H2O2 in Capan-2 cells in a dose and time dependent manner (Fig. 10). On the contrary, BITC induced a modest increase in the generation of hROS, such as singlet oxygen, superoxide, nitric oxide, hydroxyl and alkyl peroxide radicals in response to BITC treatment.

Eventually, BITC-induced ROS production substantially increased the phosphorylation of stress sensors, such as ERK (Thr202/Thy204), JNK (Thr183/Tyr185) and P38 (Thr180/Tyr182), (Fig. 11). The activation of ERK and JNK was as early as1 h after BITC treatment and was sustained until 12h. On the other hand, activation of P38 was observed around 24 h of BITC treatment (Sahu et al., 2009b).

BITC–induced ROS generation also resulted in DNA damage as evidenced by the phosphorylation of H2A.X at Ser-139, which is considered to be the hall mark of DNA double strand breaks (Sedelnikova et al., 2003). Interestingly, when BITC-treated cells were cultured in fresh medium without BITC for additional 48h cells showed persistent H2A.X phosphorylation (Fig. 12), indicating that BITC induce permanent DNA damage in Capan-2 cells (Zhang et al., 2006). As protective mechanism, DNA damage lead to cell cycle arrest to obtain brief window of time to compensate/repair the damage that occurred due to ROS

production. Accordingly, treatment of Capan-2 cells with BITC (10μM) for 24h resulted in the increased accumulation of cells in G2/M phase (42%) (Srivastava, 2004). The increased expression and phosphorylation of Chk2 (Thr-68) by BITC treatment caused G2/M arrest. Furthermore, BITC also decreased the phosphorylation and expression of Cdc25C (ser-216), Cdc2 (Tyr-15) and Cyclin B1in apan-2 cells, as compared to control cells (Fig.12).

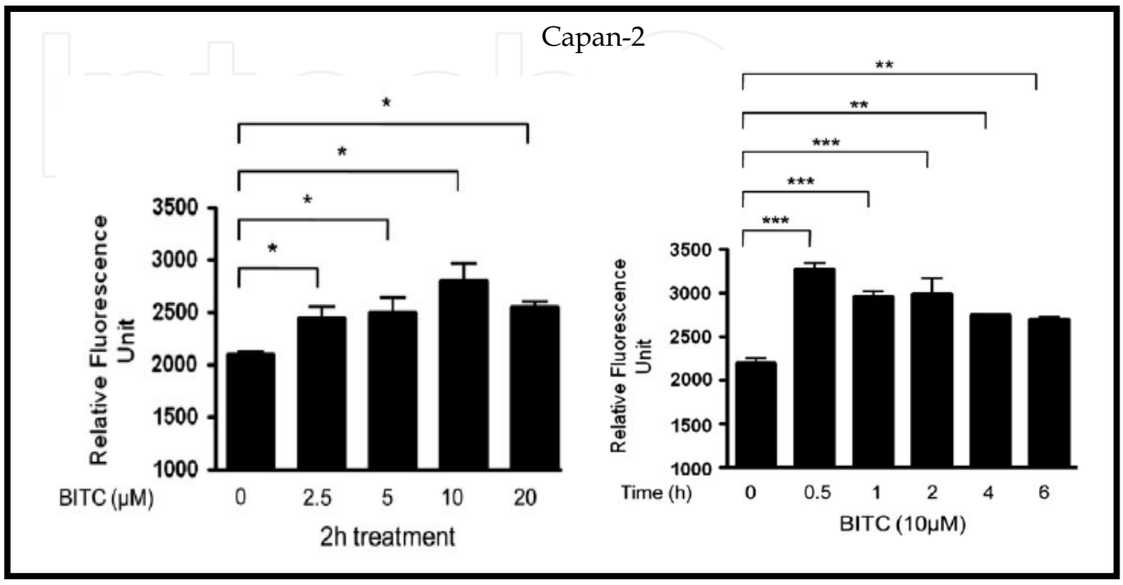


Fig. 10. BITC induces ROS generation in Capan-2 cells. (Carcinogenesis 2009: 30;1744–1753).

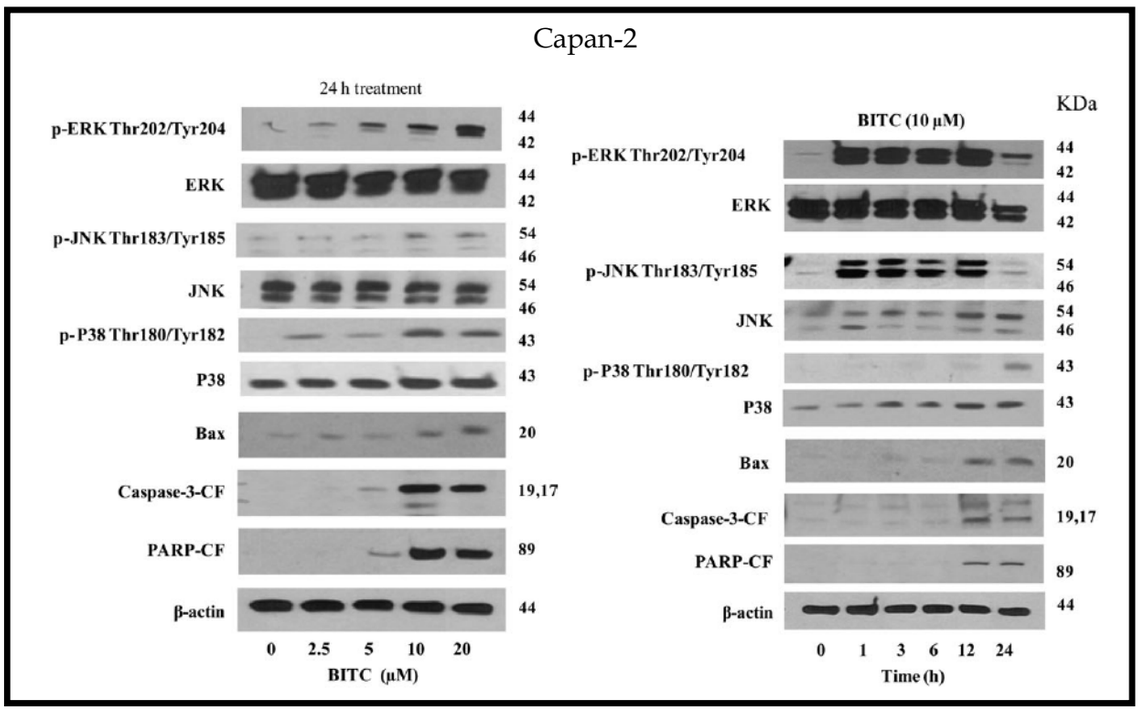


Fig. 11. BITC induces phosphorylation of MAP kinases. (Carcinogenesis 2009: 30;1744–1753).

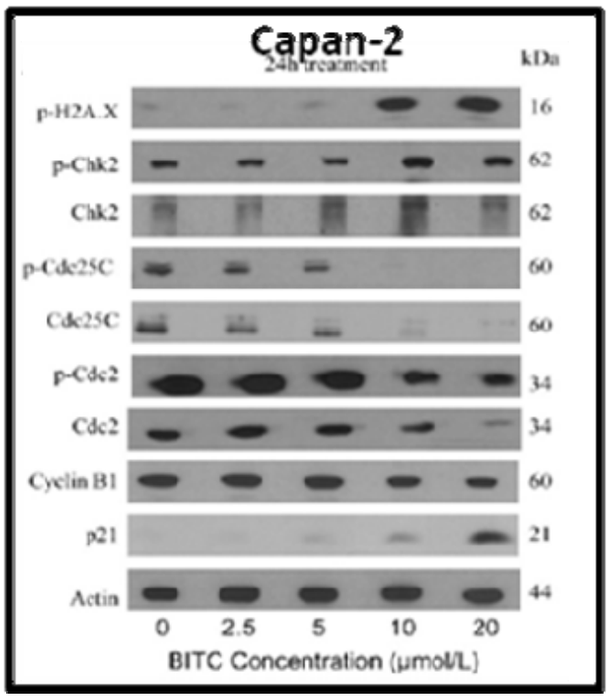


Fig. 12. Effect of BITC on cell cycle proteins. (J. Nutr. 2006: 136; 2728–2734).

Interestingly, although ERK, JNK and P38 were activated in response to BITC treatment, they had different roles in BITC-induced cell cycle arrest and apoptosis. MEK-1 inhibitor PD98059 significantly abrogated BITC induced G2/M cell cycle arrest and apoptosis (Fig. 13A, B&D). Whereas, both JNK (SP600125) and P38 (SB202190) inhibitors failed to protect the cells from BITC-mediated G2/M cell cycle arrest. Further, MEK-1 inhibitor blocked BITC-mediated activation of ERK as well as down-regulation of G2/M regulatory proteins such as cyclin-dependent kinase-1 (Cdk1), cyclin B1, Cdc25C and cleavage of caspase-3 and PARP, suggesting the involvement of ERK in BITC-induced G2/M cell cycle arrest and apoptosis (Fig. 13C). BITC-mediated apoptosis was almost completely blocked in the cells pre-treated with ERK, JNK or P38 inhibitors as evaluated by cell death apoptosis ELISA assay (Fig.13D). Similar results were obtained with MAPK8-shRNA in Capan-2 cells, indicating that all the MAPK were involved in BITC-induced apoptosis but only ERK was involved in BITC-induced cell cycle arrest.

Involvement of BITC-induced ROS generation in cell cycle arrest and apoptosis was further confirmed by treatment with antioxidants such as NAC, tiron, GSH and SOD. BITC-induced phosphorylation of MAPK and down regulation of cell cycle proteins such as GSH, Cdk1, Cdc25C, Cyclin B1 were significantly blocked by the treatment also with NAC (Fig.14). Furthermore, BITC-induced apoptosis was inhibited when cells were pre-treated with antioxidants, such as tiron, GSH and SOD. These results indicate that BITC induces ROS in pancreatic cancer cells which leads to DNA damage, cell cycle arrest and apoptosis.

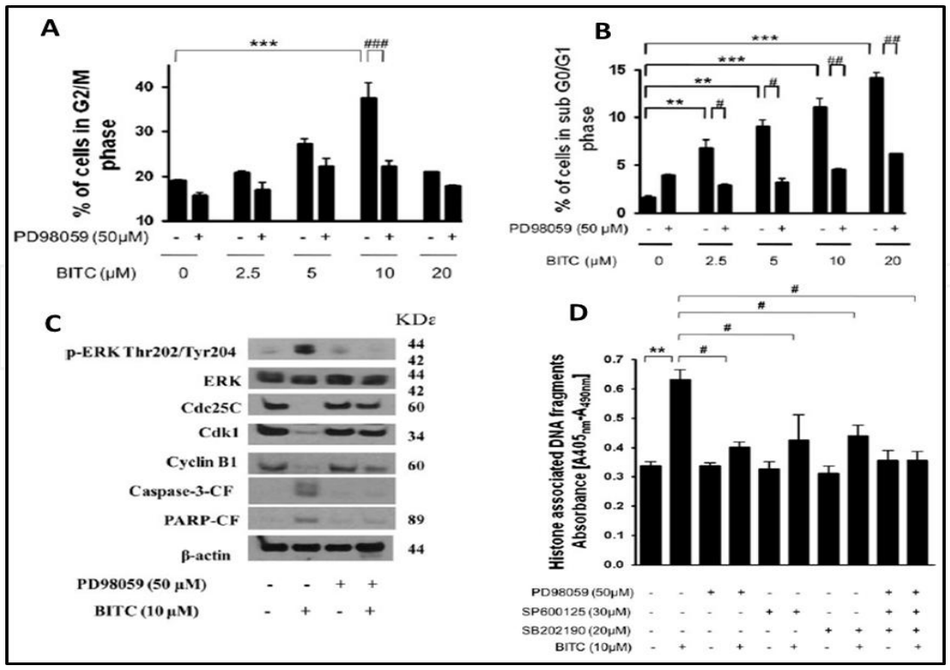


Fig. 13. MAPK inhibitors rescue pancreatic cancer cells from BITC induced apoptosis and cell cycle arrest. (Carcinogenesis 2009: 30;1744–1753).

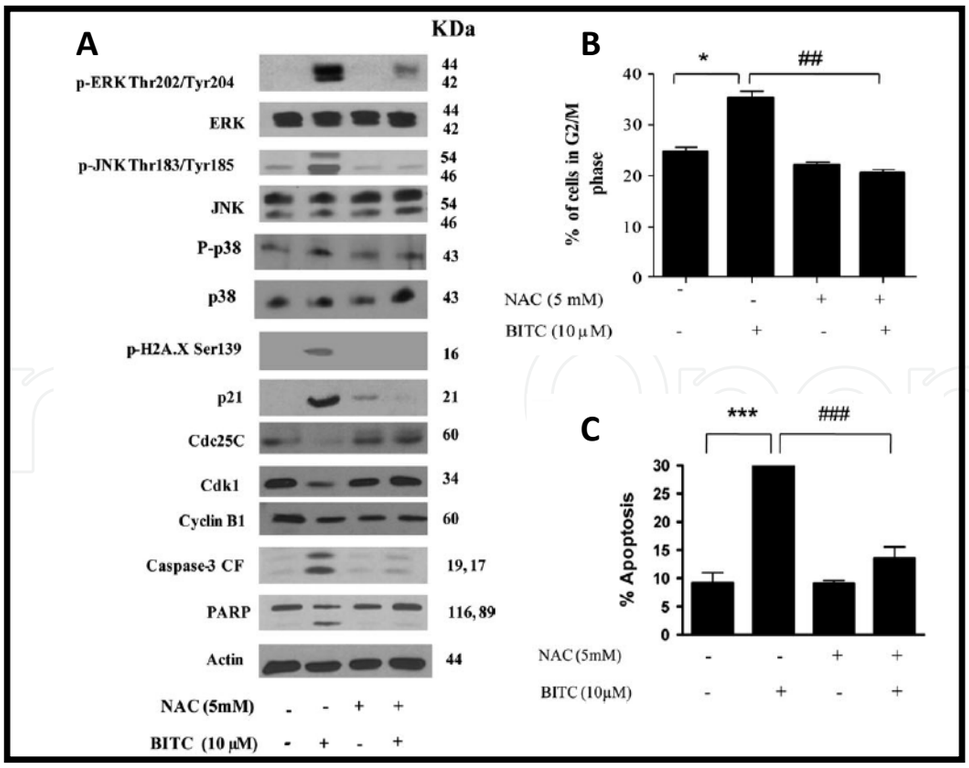


Fig. 14. Antioxidants protects Capan-2 cells from BITC-induced cell cycle arrest and apoptosis. (Carcinogenesis 2009: 30;1744–1753).

6. BITC sensitizes human pancreatic cancer cells to radiation and TRAIL treatment

Increased systemic toxicity and resistance are the major drawbacks of radiation therapy in pancreatic cancer treatment. Interestingly, BITC potentiated therapeutic effect of γ-irradiation in BxPC-3 cells. BxPC-3 cells were pre-treated with 2.5 or 5 μM BITC for 24h, followed by treatment with different doses of γ-irradiation (2.5, 5, 10 and 20Gy) at a dose of 4Gy/minute. The cells were allowed for 24 or 48h before being analyzed for survival assay. BxPC-3 cells pre-treated with BITC and treated with 5Gy γ-irradiation show intense cell death, as compared to either treatment alone, indicating that BITC sensitizes the cells to γ-irradiation (Sahu et al., 2009c). Furthermore, as shown in Table 1, more cells were accumulated in G2/M arrest in response to combination treatment, as compared to either treatment alone. In addition, expression of cell cycle proteins Chk2 and Cdc25 was increased in combination treated cells, as compared to control cells. Interestingly, DNA damage markers H2A.X (Ser-139) and ATR (Ser-428) also increased in combination treatment, indicating that BITC sensitizes cells to γ-irradiation. In agreement with cell cycle data, apoptosis induction was more in combination treated cells.

Similarly, BITC also potentiated the apoptosis inducing activity of TRAIL in pancreatic cancer cells. BxPC3 cells had a 3.84 fold increase in apoptosis upon treatment with BITC alone, an 8.65 fold increase was observed with TRAIL alone, and a 12.39 fold increase was seen when cells were treated with BITC combined with TRAIL. Similarly, Panc-1 cells underwent a 1.49 fold increase in apoptosis upon treatment with BITC, a 1.82 fold increase with TRAIL alone, and a 3.45 fold increase with BITC combined with TRAIL compared to vehicle. Interestingly, sensitization of pancreatic cancer cells to TRAIL by BITC was more in Kras wild type cells (BxPC-3) as compared to Kras mutated cells (PanC-1 and MIA PaCa-2). Further studies are needed to elucidate the role of Kras mutation in TRAIL or BITC-induced apoptosis.

7. BITC inhibits pancreatic cancer angiogenesis

Pancreatic tumors can acquire substantial development of new blood vessels in a process called angiogenesis (Philip, 2008). This vascular development is a necessary component of solid tumor growth and progression. Numerous reports have shown that disrupting tumor angiogenesis could effectively inhibit tumor growth and metastasis. BITC has shown promising potentials as anti-angiogenesis agent for pancreatic cancer *vitro* and *in vivo*.

In a rat aorta ring assay model, treatment with 5 μM BITC reduced sprouting of new blood vessels by 67% as compared to control aortic rings (Fig. 15A). Furthermore, 5 μmol BITC treatment drastically (70%) suppressed new embryonic blood vessel growth in each egg as compared to control eggs in a CAM assay model (Fig. 15B), indicating that BITC has potential to inhibit tumor angiogenesis (Boreddy et al., 2011b).

BITC was also effective in suppressing the secretion of pro-angiogenic factors from pancreatic cancer cells under both, normoxia and hypoxia conditions. Hypoxia alone induced the secretion of both MMP-2 and VEGF around 2-4 folds in both BxPC-3 and PanC-1 cells; however, BITC significantly inhibited the secretion of both VEGF and MMP-2 from the both BxPC-3 and PanC-1 cells under normoxia and hypoxia conditions (Fig. 16A-D). Interestingly, BITC significantly inhibited the migration and invasion of both, BxPC-3 and PanC-1 cells in a dose dependent manner. These steps are critical for the migration of the tumor cells *in vivo*.

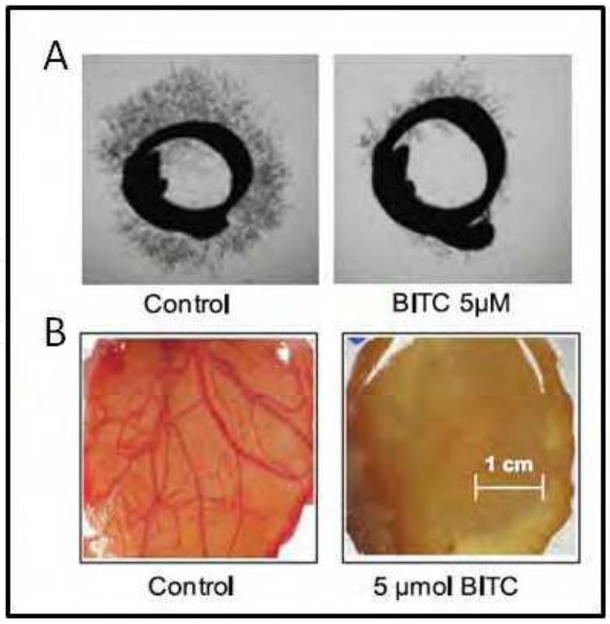


Fig. 15. BITC inhibits ex vivo angiogenesis. (PLoS ONE 2011: 6(10); e25799).

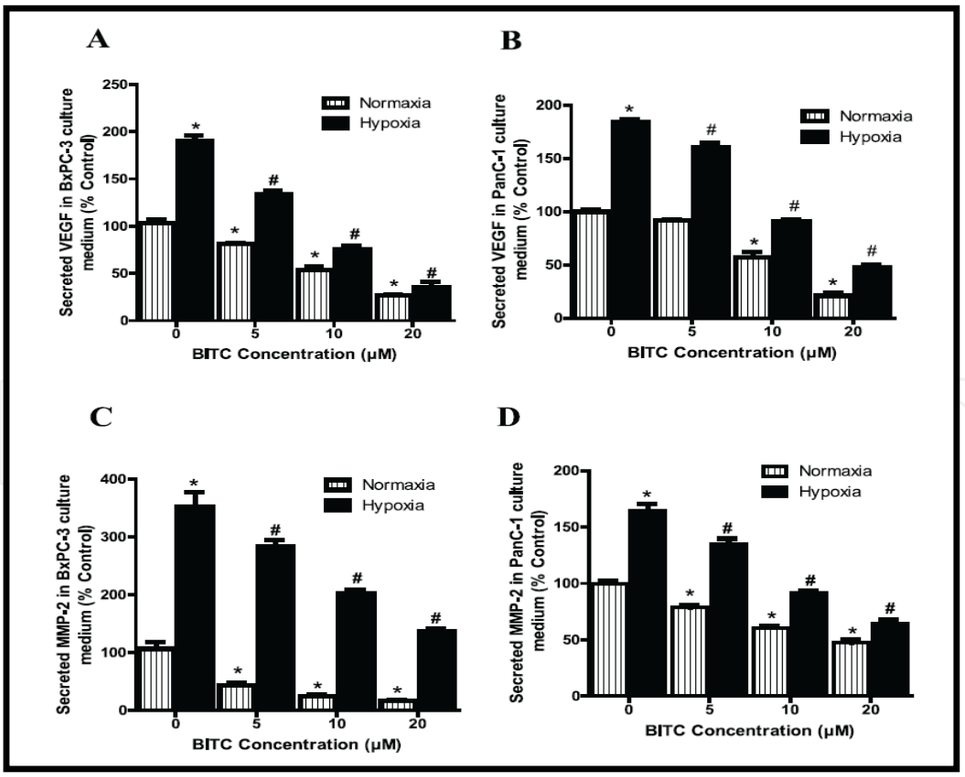


Fig. 16. BITC inhibits the secreastion of VEGF and MMP-2 in pancreatic cancer celss under both normoxia and hypoxia conditions. (PLoS ONE 2011: 6(10); e25799, 1-12).

Furthermore, BITC was quite effective in down regulating various angiogenic factors such as, HIF1-α, VEGFR-2, MMP-2, Rho A, Rho C and RAC1,2,3 in dose dependent manner in BxPC-3 and PanC-1 cells (Fig. 17A). Similarly, BITC inhibited the expression of angiogenic proteins in human endothelial cells (HUVEC) (Fig. 17B), in a dose dependent manner. Interestingly, BITC was ineffective in STAT3-overexpressing BxPC-3 cells. Furthermore, when STAT-3 was silenced in BxPC-3 cells the molecular changes were similar to that of BITC treatment changes indicating that BITC inhibits tumor angiogenesis by targeting STAT-3 (Fig. 17C&D).

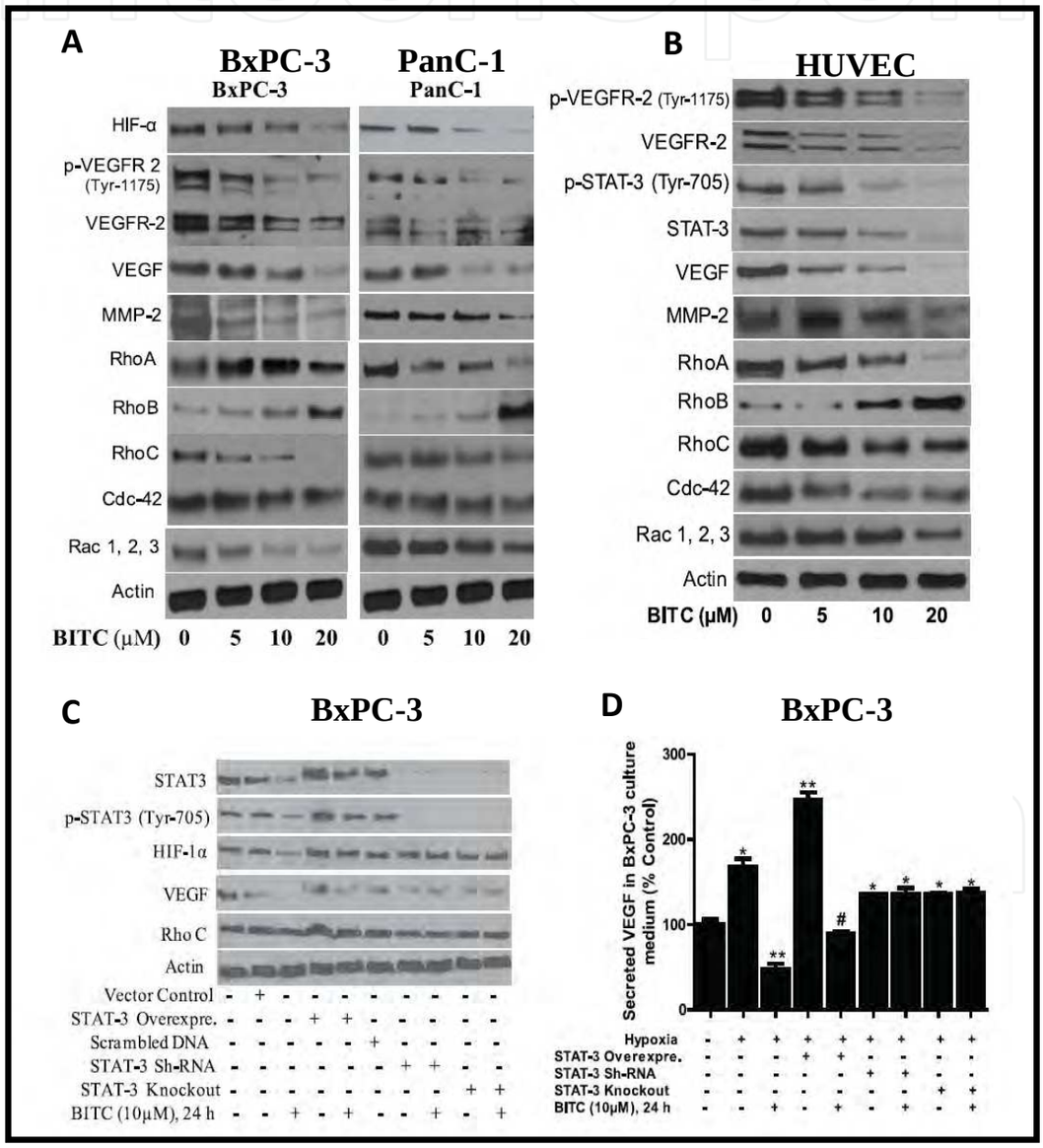


Fig. 17. BITC down regulates the critical molecules of angiogenesis in BxPC-3, PanC-1 and HUVECs by targeting the STAT3. (PLoS ONE 2011: 6(10); e25799, 1-12).

8. BITC suppresses pancreatic tumor growth and angiogenesis in vivo

BITC exhibited similar results *in vivo* as observed in culture. Tumor growth in BITC-fed mice was substantially retarded, as compared to control mice. Tumors appeared to grow more slowly in BITC-fed mice as compared with control mice. For example, 6 weeks after treatment with12 µmol BITC, the average tumor volume in control mice was about 1.92-fold higher than that in BITC-treated mice (mean tumor volume, control *vs* BITC treated: 334 vs172 mm^3, difference = 162 mm^3, 95% CI = 118 to 204 mm^3 ;P = .008; Fig.18A). Furthermore, average tumor weight in BITC-treated mice was 225mg, whereas in control mice tumor weight was 425mg (Fig. 18B), indicating that BITC potentially suppress the growth of pancreatic tumors *in vivo*. Interestingly, BITC-treated mice did not show any toxicity symptoms such as weight loss (Fig. 18C).

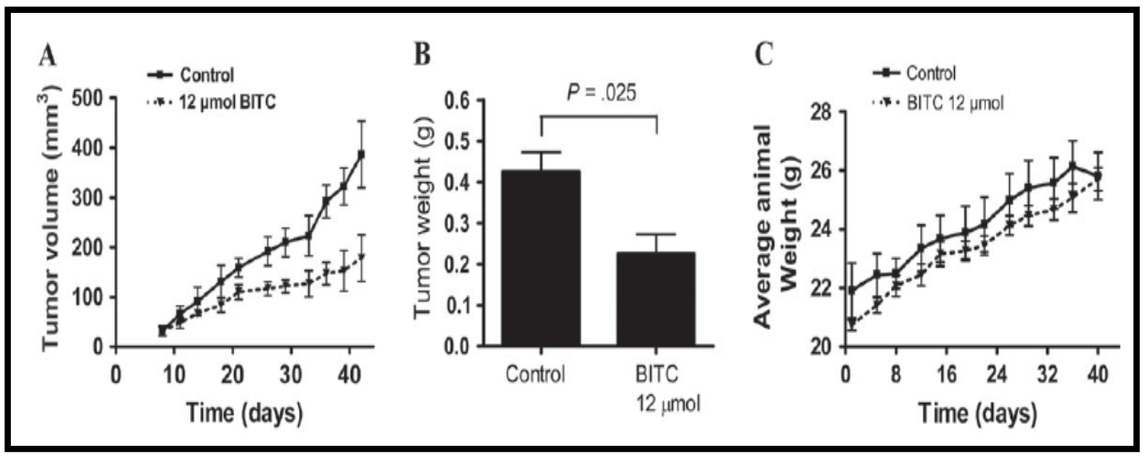


Fig. 18. BITC suppresses the growth of pancreatic cancer xenografts in vivo. (J Natl Cancer Inst 2009;101: 176-193).

It is noteworthy that when animals were orally gavaged with 12µmol/day BITC for 46 days, mean BITC concentration in plasma after1 hour of BITC administration was 6.5 ± 0.1 mmol/ L (n=10), whereas accumulated BITC concentration in the tumors after 46 days was 7.5 ± 0.3 µmol/g (n=10). These results indicate that the therapeutic concentration of BITC could be achieved *in vivo* by oral feeding.

A 76% reduction in hemoglobin content was observed in BITC-treated matrigel plugs that were implanted in Nu-Nu athymic nude mice as compared to untreated plugs (Fig. 19A). Similarly, BITC-treated tumor xenografts showed 61% reduced hemoglobin content as compared to untreated xenografts (Fig. 19B).

Tumors excised from BITC-treated mice showed reduced phosphorylation of STAT3 (Tyr-705 and Ser-727) (Fig. 20A), AKT (Ser-473 and Ser-308), FOXO1 (Ser-256) and FOXO3a (Ser-253) (Fig. 20B). Furthermore, protein expression of STAT3 and angiogenic proteins (Fig. 20C) was down regulated, whereas expression of AKT, FOXO1, FOXO3a remained unaltered. Nonetheless, Bim expression was significantly increased in BITC-treated tumor as compared to vehicle alone treated tumors indicating that the *in vivo effect* of BITC was similar to *in vitro* effects.

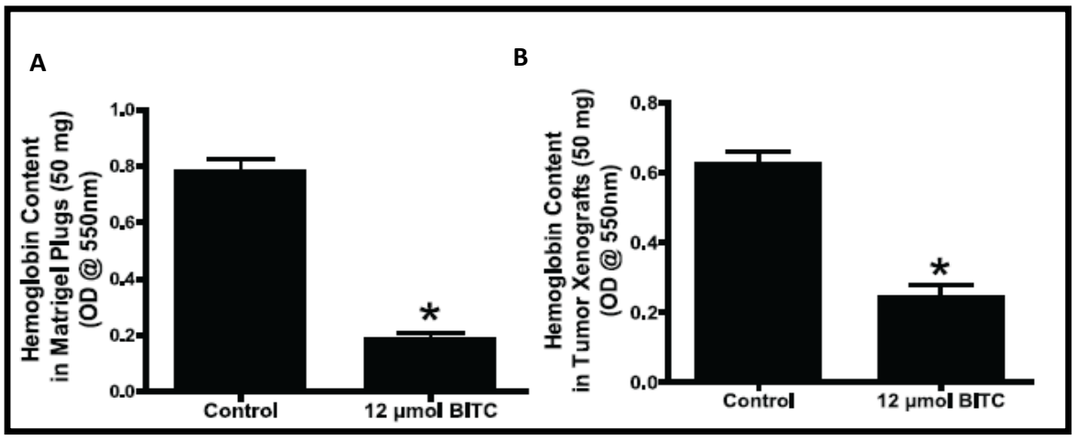


Fig. 19. BITC inhibits angiogenesis in vivo. (Clin Cancer Res 2011: 17(7); 1784–1795).

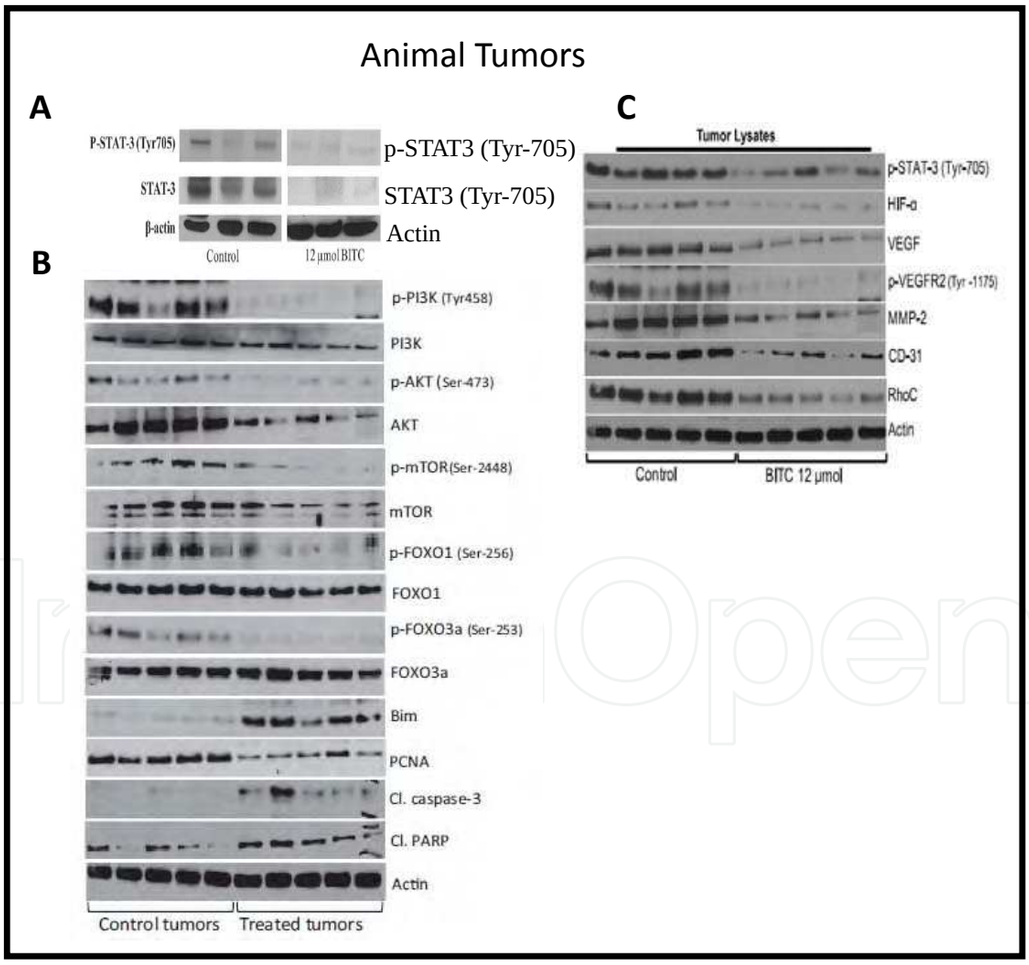


Fig. 20. BITC down regulates key molecules of survival and angiogenesis pathways. (Natl Cancer Inst 2009;101: 176-193. Clin Cancer Res 2011: 17(7); 1784–1795. PLoS ONE 2011: 6(10); e25799, 1-12).

9. Conclusion

9.1 Does BITC have multiple targets in pancreatic cancer?

Since, BITC inhibits the phosphorylation and protein levels of various key survival molecules such as STAT3, AKT and NFkB, indicating that BITC has multiple targets in pancreatic cancer. However, at this time, it is not clear whether BITC is targeting various survival pathways individually or it is the tandem effect upstream regulators. Since previous reports showed that STAT3 is being regulated by AKT through FOXO1 (Kortylewski et al., 2003) and NFkB is a direct target of AKT (Dan, 2008), presently we assume that AKT is the main target of BITC and other targets are obligated events but further studies are needed to conclude interaction of these pathways (Fig. 21).

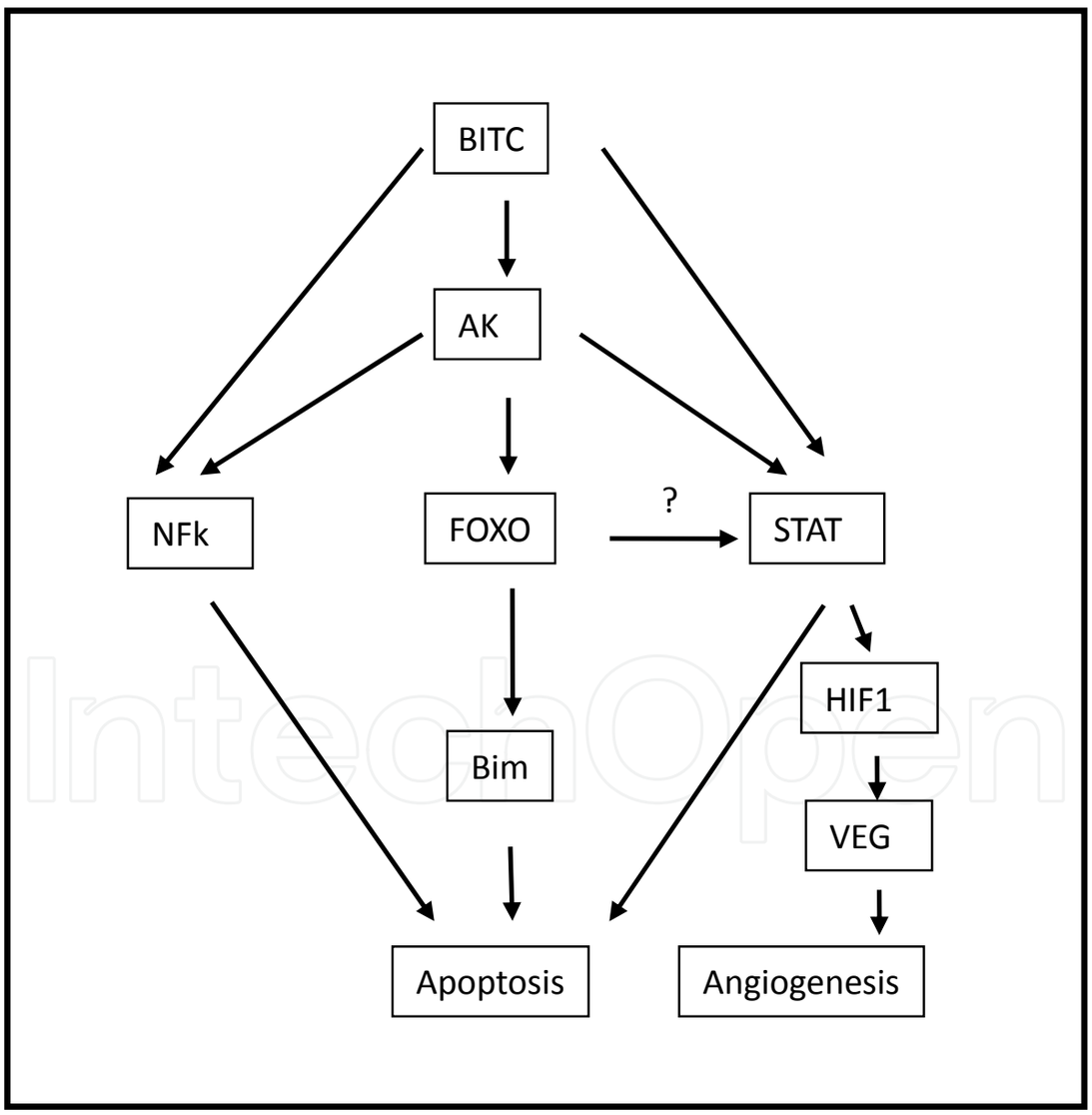


Fig. 21. BITC mechanism of action in pancreatic cancer

10. References

Arlt A, Gehrz A, Müerköster S, Vorndamm J, Kruse ML, Fölsch UR & Schäfer H. (2003). Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. *Oncogene, Vol.* 22, No. 21, pp.3243-51, ISSN 0950-9232

Batra S, Sahu RP, Kandala PK & Srivastava SK. (2010). Benzyl isothiocyanate-mediated inhibition of histone deacetylase leads to NF-kappaB turnoff in human pancreatic carcinoma cells. *Mol Cancer Ther, Vol.*9 No. 6, pp. 1596-608, ISSN 1535-7163

Berishaj M, Gao SP, Ahmed S, Leslie K, Al-Ahmadie H, Gerald EL, Bornmann W, & Bromberg JF. (2007) Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. *Breast Cancer Res,* Vol. 9, No. 3, pp. R32, ISSN 1465-5411

Boreddy SR, Pramanik KC & Srivastava SK. (2011a). Pancreatic Tumor Suppression by Benzyl Isothiocyanate Is Associated with Inhibition of PI3K/AKT/FOXO Pathway. *Clinical Cancer Research,* Vol. 17, Issue 7, pp. 1784-1795, ISSN 1078-0432

Boreddy SR, Sahu RP & Srivastava SK. (2011b). Benzyl Isothiocyanate Suppresses Pancreatic Tumor Angiogenesis and Invasion by Inhibiting HIF-α/VEGF/Rho-GTPases: Pivotal Role of STAT-3. *PLoS ONE, Vol.* 6, No. 10, pp. e25799, ISSN 1932-6203

Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C & Darnell JE Jr.(1999). Stat3 as an oncogene. *Cell,*Vol. 98, No. 3, pp. 295-303, ISSN 0092-8674

Chan KS, Sano S, Kiguchi K, Anders J, Komazawa N, Takeda J & DiGiovanni J. (2004). Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. *J Clin Invest,* Vol. 114, No. 5, pp. 720-8, ISSN 0021-9738

Chiarle R, Simmons WJ, Cai H, Dhall G, Zamo A, Raz R, Karras JG, Levy DE & Inghirami G. (2005). Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. *Nat Med,*Vol. 11, No. 6, pp. 623-9, ISSN 1078-8956

Dan HC, Cooper MJ, Cogswell PC, Duncan JA, Ting JP & Baldwin AS. (2008). Akt-dependent regulation of NF-{kappa}B is controlled by mTOR and Raptor in association with IKK. *Genes Dev,* Vol. 22, No. 11, pp. 1490-500, ISSN 0890-9369

Darnell JE. (2005). Validating Stat3 in cancer therapy. *Nat Med,* Vol. 11, No. 6, pp.595-6, ISSN 1078-8956

Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS & Reed JC. (1998). IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J,* Vol. 17, No. 8, pp. 2215-23, ISSN 0261-4189

Finkielsztein A & Kelly GM. (2009). Altering PI3K-Akt signalling in zebrafish embryos affects PTEN phosphorylation and gastrulation. *Biol Cell,* Vol. 101, No. 11, pp.661-78, ISSN 0248-4900

Ghosh S, May MJ & Kopp EB. (1998). NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol,*Vol. 16, pp. 225-60, ISSN 0732-0582

Lorraine A. Hart, Vijaya LK, Ian MA, Peter JB, & Chung KF. (1998). Activation and localization of transcription factor, nuclear factor-kappaB, in asthma. *Am J Respir Crit Care Med,*Vol. 158, No. 5, pp. 1585-92, ISSN 1073-449X

Jemal A, Siegel R, Xu J & Ward E. (2010). Cancer statistics, 2010. *CA Cancer J Clin,* Vol. 60, No. 5, pp. 277-300, ISSN 0007-9235

Kim EJ, Hong JE, Eom SJ, Lee JY & Park JH. (2011). Oral administration of benzyl-isothiocyanate inhibits solid tumor growth and lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice. *Breast Cancer Res Treat*, Vol. 130, No. 1, pp. 61-71, ISSN 0167-6806

Kortylewski M, Feld F, Krüger KD, Bahrenberg G, Roth RA, Joost HG, Heinrich PC, Behrmann I & Barthel A. (2003). Akt modulates STAT3-mediated gene expression through a FKHR (FOXO1a)-dependent mechanism. *J Biol Chem*, Vol. 278, No. 7, pp. 5242-5249, ISSN 0021-9258

Kreuz S, Siegmund D, Scheurich P & Wajant H. (2001). NF-kappaB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. *Mol Cell Biol,*Vol. 21, No. 12, pp. 3964-3973, ISSN 0270-7306

Obexer P, Geiger K, Ambros PF, Meister B & Ausserlechner MJ. (2007). FKHRL1-mediated expression of Noxa and Bim induces apoptosis via the mitochondria in neuroblastoma cells. *Cell Death Differ,* Vol. 14, No. 3, pp. 534-47, ISSN 1350-9047.

Osaki M, Oshimura M & Ito H. (2004). PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis,* Vol. 9, No. 6, pp. 667-76, ISSN 1360-8185

Philip PA. (2008). Targeting angiogenesis in pancreatic cancer. *Lancet*, Vol. 371, No. 9630, pp. 2062-4, ISSN 0023-7507.

Sahu RP & Srivastava SK. (2009a). The role of STAT-3 in the induction of apoptosis in pancreatic cancer cells by benzyl isothiocyanate. *J Natl Cancer Inst,* Vol. 101, No. 3, pp. 176-93, ISSN 0027-8874

Sahu RP, Zhang R, Batra S, Shi Y, Srivastava SK. (2009b). Benzyl isothiocyanate-mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of MAPK in human pancreatic cancer cells. *Carcinogenesis,* Vol. 30, No. 10, pp. 1744-53, ISSN 0143-3334

Sahu RP, Epperly MW & Srivastava SK. (2009c). Benzyl isothiocyanate sensitizes human pancreatic cancer cells to radiation therapy. *Front Biosci (Elite Ed),* Vol. 1, pp. 568-76, ISSN 1093-9946

Salih DA & Brunet A. (2008). FoxO transcription factors in the maintenance of cellular homeostasis during aging. *Curr Opin Cell Biol,* Vol. 20, No. 2, pp. 126-36, ISSN 0955-0674

Sarbassov DD, Guertin DA, Ali SM & Sabatini DM. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science,* Vol. 307, No. 5712, pp. 1098-101, ISSN 0036-8075

Sawai H, Yasuda A, Ochi N, Ma J, Matsuo Y, Wakasugi T, Takahashi H, Funahashi H, Sato M & Takeyama H. (2008). Loss of PTEN expression is associated with colorectal cancer liver metastasis and poor patient survival. *BMC Gastroenterol,* Vol. 8, pp. 56, ISSN 1471-230X

Schlieman MG, Fahy BN, Ramsamooj R, Beckett L & Bold RJ. (2003). Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. *Br J Cancer,* Vol. 89, No. 11, pp. 2110-2115, ISSN 0007-0920

Sedelnikova OA, Pilch DR, Redon C & Bonner WM. (2003). Histone H2AX in DNA damage and repair. *Cancer Biol Ther,* Vol. 2, No. 3, pp. 233-235, ISSN 1538-4047

Shou Y, Li N, Li L, Borowitz JL & Isom GE. (2002). NF-kappaB-mediated up-regulation of Bcl-X(S) and Bax contributes to cytochrome c release in cyanide-induced apoptosis. *J Neurochem,* Vol. 81, No. 4, pp. 842-52, ISSN 0022-3042

Srivastava SK & Singh SV. (2004). Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis,* Vol. 25, No. 9, pp. 1701-9, ISSN 0143-3334

Stoner GD & Morse MA. (1997). Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. *Cancer Lett*, Vol. 114, No. 113-119 ISSN 0304-3835

Sun Z & Andersson R. (2002). NF-kappaB activation and inhibition: a review. *Shock,* Vol. 18, No. 2, pp. 99-106, ISSN 1073-2322

Tzivion G, Dobson M & Ramakrishnan G. (2011). FoxO transcription factors; Regulation by AKT and 14-3-3 proteins. *Biochim Biophys Acta,* Vol. 1813, No. 11, pp. 1938-45, ISSN 0006-3002

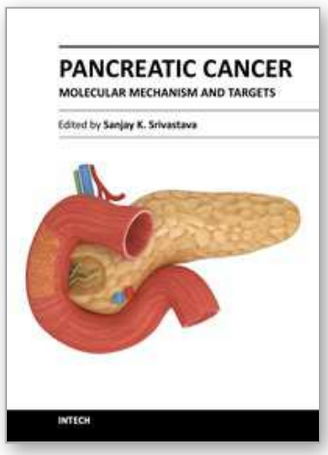
Vivanco I & Sawyers CL. (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer,* Vol. 2, No. 7, pp. 489-501, ISSN 1474-175X

Wei D, Le X, Zheng L, Wang L, Frey JA, Gao AC, Peng Z, Huang S, Xiong HQ, Abbruzzese JL & Xie K. (2003). Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene,* Vol. 22, No. 3, pp. 319-29, ISSN 0950-9232

Zhang R, Loganathan S, Humphreys I & Srivastava SK. (2006). Benzyl isothiocyanate-induced DNA damage causes G2/M cell cycle arrest and apoptosis in human pancreatic cancer cells. *J Nutr,* Vol. 136, No. 11, pp. 2728-34, ISSN 0022-3166

Zhang Y, Talalay P, Cho CG & Posner GH. (1992). A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci U S A,* Vol. 89, No. 6, pp. 2399-403, ISSN 0027-8424

IntechOpen



Pancreatic Cancer - Molecular Mechanism and Targets
Edited by Prof. Sanjay Srivastava

ISBN 978-953-51-0410-0
Hard cover, 432 pages
Publisher InTech
Published online 23, March, 2012
Published in print edition March, 2012

This book provides the reader with an overall understanding of the biology of pancreatic cancer, hereditary, complex signaling pathways and alternative therapies. The book explains nutrigenomics and epigenetics mechanisms such as DNA methylation, which may explain the etiology or progression of pancreatic cancer. Book also summarizes the molecular control of oncogenic pathways such as K-Ras and KLF4. Since pancreatic cancer metastasizes to vital organs resulting in poor prognosis, special emphasis is given to the mechanism of tumor cell invasion and metastasis. Role of nitric oxide and Syk kinase in tumor metastasis is discussed in detail. Prevention strategies for pancreatic cancer are also described. The molecular mechanisms of the anti-cancer effects of curcumin, benzyl isothiocyante and vitamin D are discussed in detail. Furthermore, this book covers the basic mechanisms of resistance of pancreatic cancer to chemotherapy drugs such as gemcitabine and 5-flourouracil.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Srinivas Reddy Boreddy, Kartick C. Pramanik and Sanjay K. Srivastava (2012). Molecular Targets of Benzyl Isothiocyanates in Pancreatic Cancer, Pancreatic Cancer - Molecular Mechanism and Targets, Prof. Sanjay Srivastava (Ed.), ISBN: 978-953-51-0410-0, InTech, Available from: http://www.intechopen.com/books/pancreatic-cancer-molecular-mechanism-and-targets/molecular-targets-of-benzyl-isothiocyanates-in-pancreatic-cancer-

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen