Chapter 10

Apoptotic Molecular Advances in Breast Cancer Management

Pontsho Moela and Lesetja R. Motadi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61654

Abstract

Breast cancer is the most common cancer type amongst women, accounting for most female cancer deaths second to cervical cancer worldwide. It is, therefore, highly crucial to understand the molecular biology and explore other pathways involved in carcinogenesis in order to select appropriate treatment not only for breast cancer but for other cancers as well. Cancer progression is favoured by DNA damage and in most cases a consequent disruption of the apoptotic pathway, thus leading to uncontrolled cell proliferation. Therefore, current therapeutic strategies aim at targeting the apoptotic pathways in order to combat cancer. In this manuscript, we discuss the ways in which evasion of apoptosis during carcinogenesis occurs and the types of current therapeutic strategies as well as promising future approaches against breast cancer.

Keywords: Breast cancer, apoptosis, small molecules, p53, RBBP6

1. Introduction

The human body is composed of trillions of cells that behave and function to provide structure of the body, convert nutrients into energy and carry out specialised functions [1, 3]. Growing, dividing, differentiating and dying are the cells' behavioural mechanisms to maintain tissue homeostasis [3]. However, molecular disturbances that disrupt this balance may potentially lead to disease. Such molecular disturbances include mutations, among others, during which any change to the DNA sequence might result in abnormality in the cell or tissue [4]. With a population of more than a trillion cells, the human body is prone to mutations that may give one cell a selective advantage of growing and dividing more vigorously to become a growing mutant clone [4, 5]. Such mutations, in which a mutant clone of cells grows and divides out of control at an expense of neighbouring wild-type cell populations, serve as a prerequisite for the development of cancer [3].

Cancer is defined as uncontrolled cell proliferation that leads to the formation of abnormal cells and invasion of other adjacent tissues [1, 3-5]. The migration of cells from the origin of tumour to another part of the body is referred to as metastasis. Tumours can either be malignant or benign. While malignant tumours have the ability to invade surrounding tissue, benign tumours cannot invade other tissues and are therefore not as life-threatening [3]. Efficient treatment against malignant tumours is therefore necessary in cancer management. In this chapter, we discuss current anticancer strategies that are targeted on the apoptosis pathway in breast cancer management.

In order to understand breast cancer, it is necessary to understand the normal anatomy of the female breast [3, 20]. The female breast is made up of milk-producing glands called lobules which are connected to ducts that transport milk from the glands to the nipples. The ducts and lobules are surrounded by connective tissue, fatty tissue, blood vessels and lymphatic vessels. In most cases, breast cancer starts in cells surrounding the ducts or the lobules [23]. Metastatic breast cancer is as a result of migration of cancerous cells from ducts and/or lobules via lymphatic vessels to the lymph nodes of the lymphatic system [3, 20, 23].

Breast cancer is the most common cancer type amongst women accounting for many cancer deaths, second to cervical cancer. Risk factors of breast cancer are divided into non-modifiable and modifiable factors [39]. Advanced age, female gender, menarche before the age of 12, menopause after the age of 45, genetic mutations and family history are the major non-modifiable risk factors associated with breast cancer [6, 11, 26, 46, 56, 57]. Breast cancer risk factors that can be controlled include hormone replacement therapy, oral contraceptives, pregnancy, breast feeding and high breast density [31]. Behavioural and life-style risk factors associated with the development of breast cancer include poor diet, i.e. high fat, low vegetable/fruit, low fibre and high in simple carbohydrates; overweight and obesity; and decreasing physical activity [29, 39].

Nearly 80% of human breast cancers are hormone-positive (estrogen and progesterone), followed by human epidermal growth factor receptor 2 (HER2)-positive, then vascular endothelial growth factor (VEGF)-positive breast tumours [8, 9]. Targeting estrogen receptor (ER) pathway, VEGF and HER2 are the long-established breast cancer therapeutic approaches responsible for the improvements of breast cancer prevention and treatment. However, resistance to these endocrine and cell-growth-inhibiting treatments is the main drawback that reduces the benefits of these novel treatment approaches [8, 9, 20, 23]. It is therefore highly crucial to understand the molecular biology and explore other pathways involved in carcinogenesis in order to select appropriate treatment not only for breast cancer but for other cancers as well. In this chapter we discuss different ways of targeting apoptosis in breast cancer management.

2. Targeting apoptosis in breast cancer treatment

During the process of breast cancer progression, normal cells transform into malignant types as a result of genetic alterations [12]. This leads to dysregulation of cellular processes such as

http://dx.doi.org/10.5772/61654

angiogenesis, cell cycle and apoptosis [17]. Therefore, current therapeutic strategies aim at targeting these pathways, more especially apoptosis, in order to combat cancer [18]. Apoptosis is a form of programmed cell death in which cells are programmed to die if found to be cellular damaged [21, 25]. Apoptosis is made up of two major pathways called the death receptor pathway and the mitochondrial pathway, which are both propagated by a caspase cascade that ultimately leads to apoptosis induction [27, 34]. Evasion of apoptosis during carcinogenesis occurs by three distinct mechanisms: disrupted signalling of death receptors, loss of caspase activity as well as impaired balance between anti-apoptotic and pro-apoptotic proteins [14, 42, 50, 59]. Targeting the caspase cascade, Bcl-2 family proteins as well as other factors associated with apoptosis signalling have thus become the major strategy in anticancer therapeutics (table 1).

Reagent	Target	Technology	Function	Status
Apoptin	Caspases in the extrinsic pathway	Vector-based (adenoviral and virus vectors)	Caspase 3 and 8 activation	Preclinical
Flavipirodol, gossypol, depsipeptide, ABT-737, ABT-264, fenretinide, HA 14-1, GX15-070	Anti-Bcl-2 family proteins	Small molecule	Inhibit BCl-2 family proteins by reducing their expression	Phase I/II
ABT 737	Anti-apoptotic proteins	Small molecule	Inhibit expression of anti-apoptotic proteins such as Bcl-xL, Bcl-2 and Bcl-W	Phase I
Oblimersen Sodium	Anti-Bcl-2 targeted drug	Antisense	Bcl-2 antisense increases survival rates in chronic myeloid leukaemia patients when combined with chemotherapy	Phase II
ONYX-015 drug	p53-based gene therapy	Adenoviral	Genetically engineered adenovirus that has been modified to infect and lyse p53-deficient cells	Phase III
$CD8^+$ cytotoxic T-lymphocytes (CTLs)	Tumour associated antigens (mutant p53)	Vaccine	Recognize TAA-derived peptides that are processed and presented on the tumours cell surface in association with MHC class I molecules, leading to killing of tumour cells	Phase I

Reagent	Target	Technology	Function	Status
Phikan083, CP-31398	p53-targeted	Small molecule	Restores p53 function by intercalating with p53-bound DNA and destabilising the p53-DNA interaction	Phase I/II
Tenovins, Nutlins, MI-219	p53-MDM2 interaction	Small Molecule	Interrupt the p53-MDM2 interaction to prevent inactivation of p53 by MDM2	Phase I/II
siMDM2, siE6/7, siBBP6	p53-MDM2, p53-E6, p53-RBBP6 interaction	Liposomal encapsulated synthetic siRNA	Interrupt p53 interaction with its negative regulators	Research

Table 1. Apoptosis-based anticancer drugs in development

2.1. Caspase-targeted therapy

Pathogenic as they are, disruptions in the apoptotic pathway provide compelling possible strategies for the treatment of breast cancer and other related types of cancers [59]. Therapeutic agents designed to re-establish the normal functioning of the apoptotic signalling pathways have the potential to get rid of over 50% of human cancers including breast cancer [34]. Novel drug discoveries in recent years have led to promising advances in the treatment of breast cancer as well as other cancers. For example, the caspase-targeting therapies that use small molecules to act as caspase activators have been identified [24, 32]. These small molecule caspase activators are pro-apoptotic due to their characteristic arginine-glycine-aspartate motif that enables them to directly convert non-active procaspase-3 into active caspase-3 thus leading to apoptosis induction.

Apoptotin is a caspase-based drug therapy that has the ability to induce caspase activity thus increasing apoptosis induction [32]. MCF-7 breast cancer cells completely lack the expression of caspase-3 due to frame-shift mutation in exon 3 of the caspase-3 gene [13]. As a result, caspase-based gene therapy that relies on caspase-3 gene delivery techniques in order to up-regulate caspase-3 expression in caspase 3-deficient breast cancers has been invented. In human liver tumorigenesis, caspase-3 gene therapy led to a significant increase in apoptosis and shrinkage in tumour size when combined with other chemotherapeutic drugs [13, 32]. Caspase-8 expression has also been found to be impaired due to hypermethylation in several cancer cells. In small cell lung carcinomas, demethylation treatments have been shown to sensitise these cancer cells to drug-induced apoptosis [32, 53].

2.2. Anti-Bcl-2 therapy

The mitochondrial pathway is down-regulated by the anti-apoptotic Bcl-2 family proteins [19, 22, 40, 43, 60]. Drug-based therapy using anti-Bcl-2 small molecules has led to a significant

http://dx.doi.org/10.5772/61654

induction of apoptosis in several cancers. Flavipirodol, gossypol, depsipeptide, ABT-737, ABT-264, fenretinide, HA 14-1 and GX15-070 are some of the small molecules that inhibit BCl-2 by reducing their expression [41, 45, 59]. Small molecules with the ability to mimic pro-apoptotic or anti-apoptotic BH3-only Bcl-2 family proteins in order to induce apoptosis have also been designed [2, 41]. This class of drugs that imitate BH3-only pro-apoptotic and anti-apoptotic Bcl-2 family proteins is referred to as BH3-only mimetic drugs [2, 35]. ABT 737 is one example of the BH3-only mimetics that has been shown to inhibit expression of anti-apoptotic proteins such as Bcl-xL, Bcl-2 and Bcl-W; and is showing promising results in clinical trials [2, 59]. The first anti-Bcl-2 targeted drug to enter clinical trials in leukemic patients is known as oblimersen sodium [41, 59]. This Bcl-2 antisense has been shown to increase survival rates in chronic myeloid leukaemia patients when combined with chemotherapy [41, 59].

2.3. p53-based gene therapy

The loss of p53 function is a common feature in almost all human cancer including breast cancer [37, 43, 47]. Because of this, there is a lot of interest in targeting p53 for anticancer therapeutic drugs [7, 10, 16, 55]. The first biological approach which is now widely used in targeting p53 is gene delivery of wild-type p53 into tumour cells using adenoviral or retroviral techniques [28, 48]. p53-based gene therapy is however not effective on its own in killing cancer cells and for this reason combinational therapies involving other modes of treatments in the presence of p53 therapy are being investigated [10, 55, 59].

For example, it was discovered that concurrent treatment using adenoviral-mediated wild-type p53 injection with ionising therapy significantly reduces tumour size in cancers of prostate, brain and spine as well as head and neck [28, 59]. Elimination of p53-defective cells using synthetic viruses designed to infect and kill cancer cells is another breakthrough in p53-based gene therapy [28, 48, 59]. One example is the ONYX-015 drug, which is a genetically engineered adenovirus that has been modified to infect and lyse p53-deficient cells [28]. Genetic alterations that take place in p53 during tumorigenesis can trigger the immune responses in both T- and B-cells [10]. This provides yet another interesting platform for p53-based anticancer therapy, and a number of p53-based vaccines are currently undergoing clinical trials [10, 59].

2.4. Small molecule approach in p53-based drug therapy

In comparison to large biological drugs that are present with complex structures, small molecular drugs are organic compounds designed to be extremely low in molecular weight and are made up of well-defined chemical structures that enable them to pass through the cell membrane when taken orally. A further advantage of small molecule drugs over biologics is that they are stable, mostly non-immunogenic and it is easy to characterise their molecular composition and heterogeneity. The mode of action for small molecules relies on their binding to specific biopolymers such as proteins and nucleic acids and act as effectors to alter function or activity of the specific biopolymer.

In cancer, small molecules are used to restore mutated proteins back to their wild-type forms and induce activity of proteins responsible for elimination of tumorigenic cells [30]. In p53-based drug therapy, several small molecules that can restore the function of mutated p53 have been investigated. One example of a small molecule drug known is CP-31398; which has been shown to restore p53 function by intercalating with p53-bound DNA and destabilising the p53-DNA interaction [30]. Another small molecule called Phikan083, which is a derivative of carbazole, has been identified as one of the small molecules that has the ability to restore mutant p53 too. The most advanced of these small molecules are those that act by interrupting the p53-MDM2 interaction which is responsible for the inactivation of wild-type p53 [51, 52, 54]. These include the nutlins, tenovins and the MI-219 [52]. MDM2 acts as a negative regulator of p53 by binding to and inactivating the function of p53. This activity results in the loss of p53-mediated apoptosis in cancer cells, thus promoting carcinogenesis. While the MI-219 small molecular drugs are responsible for the destabilisation of the MDM2-p53 interactions in order to selectively induce apoptosis and inhibit apoptosis, nutlins disrupt the MSM2-p53 complex and selectively induce senescence [51, 52, 54].

2.5. siRNA-based p53 therapy

There are certain cancers with no mutations in p53 but in which non-mutated p53 might be down-regulated by certain p53 negative regulators [30]. In these cancers, development of specific siRNAs for silencing of the negative regulatory genes is often used to activate p53 [10, 30, 55]. MDM2 E3 ligase and the viral E6 protein are two extensively studied negative regulators of p53 that are associated with cancer progression [51, 52, 54].

Under normal cellular conditions, p53 tumour suppressor gene is kept under tight regulation by the MDM2-p53 auto-regulatory feedback loop [36, 51, 52]. In response to stress stimuli such as DNA damage or radiation, activated p53 interacts with genes responsible for the induction of cell cycle arrest or apoptosis (figure 1) [36]. During cancer development, the interaction between p53 and MDM2 mediates p53 interaction with the ring finger domain of the MDM2 ubiquitin ligase for degradation of the p53 tumour suppressor protein [54]. This event compromises the occurrence of cell cycle arrest and p53-mediated apoptosis and facilitates abnormal cell proliferation[52]. The use of MDM2-specific siRNA to disrupt the p53-MDM2 interaction in breast cancer cells has been shown to induce apoptosis, inhibit cell proliferation and lead to decreased tumour size [36, 51, 52, 54].

The E6 viral protein is another thoroughly studied p53 negative regulator in HPV (human papillomavirus)-related cancers such as anogenital, cervical, head and neck cancers. During HPV infection, E6 protein expression increases in order to facilitate HPV replication and viral integration into the host cell. The E6 protein achieves this outcome by using its E3 ligase Hect domain to bind to and degrade the cellular tumour suppressor proteins p53 and pRB, thus abrogating the host cells' potential to initiate cell cycle arrest and apoptosis. Therapeutic strategies to disrupt E6-p53 interactions in the form of antisense and siRNA application specific to E6 viral protein have received the most attention in HPV-related cancer therapeutics [10, 30, 55].

A third ubiquitous protein suspected to be yet another negative regulator of active p53 especially in breast cancer progression is known as retinoblastoma binding protein 6 (RBBP6)

http://dx.doi.org/10.5772/61654

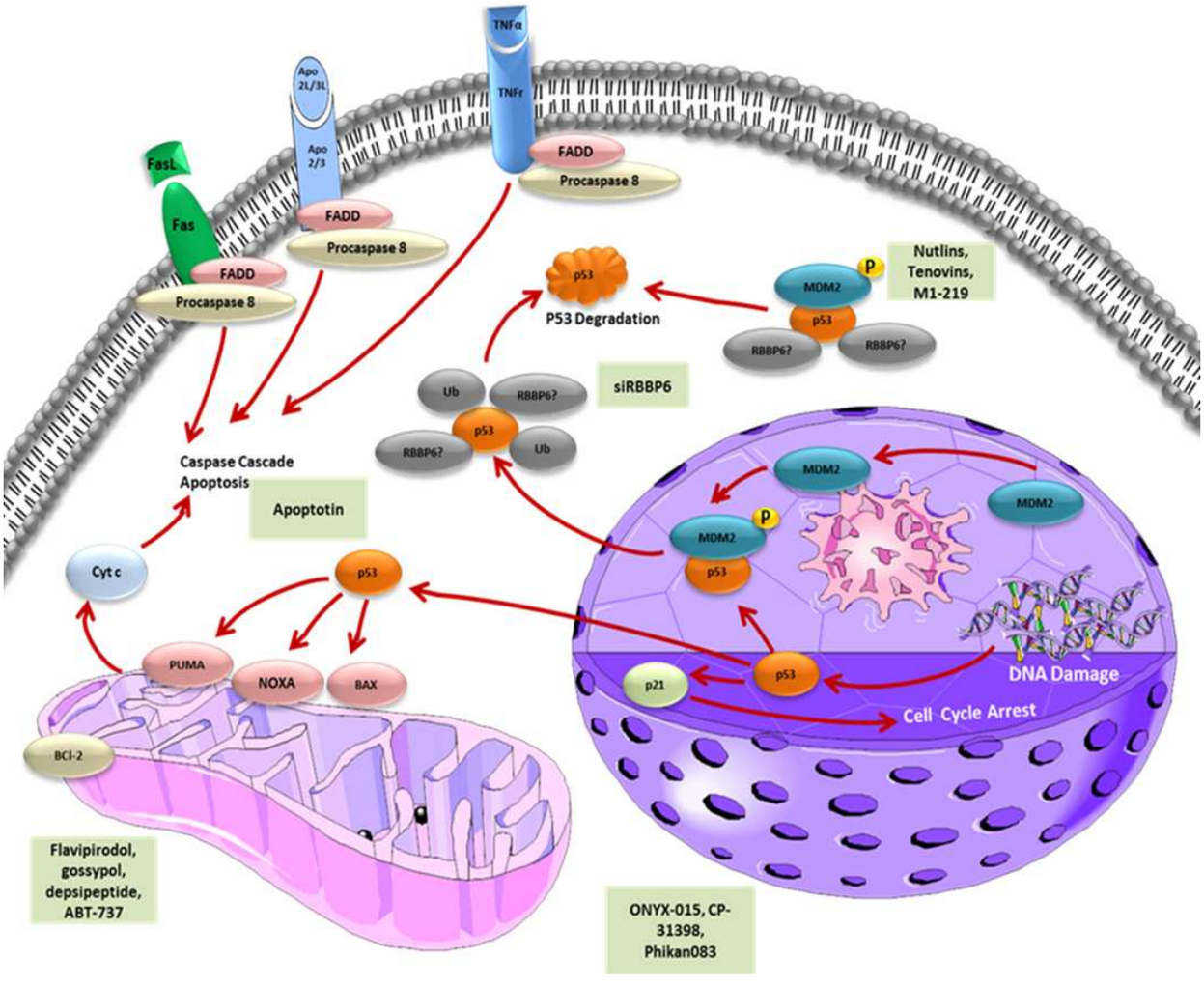

Figure 1. A simplified diagrammatic representation of the apoptotic signalling pathway and p53 negative regulation by MDM2 and another ubiquitous protein (RBBP6) suspected to be involved in p53 degradation. Current drugs that target different points of the apoptotic pathways are highlighted in light-green

[36]. RBBP6 is a 250kDa protein that has been shown to interact with and possibly lead to the degradation of p53 tumour suppressor gene since it possesses an E3 ligase activity [44, 49]. Its mRNA codes for a p53-binding domain as well as other domains known as DWNN domain, zinc finger domain and a ring finger domain, which are responsible for the ubiquitous nature of RBBP6 [44]. Besides the p53 domain, which is only present in human RBBP6, the above-mentioned domains are conserved in about all eukaryotic organisms such as humans, plants, protozoa, fungi, microsporidia and the single-celled parasite *Encephalitozoon cuniculi* [44]. RBBP6 is a spliced-associated protein and therefore exists in different other homologues known as PACT and P2P-R [44, 58].

A critical insight into the role played by RBBP6 in certain cancers via p53 has been elucidated [15]. Transfection of lung cancer cells with siRBBP6 led to a decrease in RBBP6 expression whereas sip53 transfection led to an increase in RBBP6 expression and, according to this study, RBBP6 may be involved in the degradation of p53 thereby enhancing abnormal cell prolifer-

ation [38]. In one study, it was demonstrated that down-regulation of the PACT homologue of RBBP6 in mice induces embryonic lethality with a consequent accumulation of p53 and a widespread apoptosis [33]. In addition to identifying PACT as a negative regulator of p53, further discoveries suggest that PACT knockdown enhances p53-Hdm2 interaction thus reducing p53 poly-ubiquitination by RBBP6 [33].

In recent studies, it was found that silencing RBBP6 gene in MCF-7 and CAMA-1 cells led to p53 up-regulation and sensitised the breast cancer cells to apoptosis induction [36]. Concurrent treatment of these cells with apoptosis-inducing agents, camptothecin or staurosporine, further increased apoptosis induction [36]. Furthermore, up-regulation of bax as a result of the co-treatment provided early insights into the possible mechanism behind the observed apoptosis [36]. Taken together, it is suspected that RBBP6 silencing may be responsible for the identified p53 up-regulation in breast cancer and other cancers and that the observed apoptosis is more likely p53-dependent; however, further *in vivo* investigations would validate these observations.

3. Conclusions

Taken all together, it is evident that anticancer therapeutics primarily depend on apoptosis pathway activation in breast cancer and several other cancers. However, a few milestones still need to be reached with regard to this novel anti-tumour molecular approach. For example, most of the experimentally studied apoptosis-inducing regimens in breast cancer cells have not reached the clinical stages. Another important factor that needs to be addressed in apoptosis-targeted therapy is to determine whether the observed cytotoxicity of breast cancer cells in experimental settings is comparable in clinical settings. Moreover, understanding tumour biology of individual cancer patients can help select therapeutic interventions that are highly specific to a presented tumour. Nonetheless, the link between apoptosis and tumorigenesis has been thoroughly investigated in breast cancer and has led to lots of promising strategies that attempt to eradicate cancer cells by targeting the apoptosis signalling pathway.

Author details

Pontsho Moela[1] and Lesetja R. Motadi[2*]

*Address all correspondence to: lesetja2007i@webmail.co.za

1 School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa

2 Department of Biochemistry, North-West University (Mafikeng Campus), Potchefstroom, Sout, Africa

http://dx.doi.org/10.5772/61654

References

[1] Abeloff M.D., Wolff A.C., Weber B.L., et al. (2008) Cancer of the Breast. In: Abeloff M.D., Armitage J.O., Lichter A.S., et al., eds. *Clinical Oncology*. 4th ed. Philadelphia. Elsevier; 1875-1943

[2] Albershardt T.C., Salerni B.L., Soderquist R.S., Bates D.J., Pletnev A.A., Kisselev A.F. and Eastman A. (2011) Multiple BH3 mimetics antagonize anti-apoptotic MCL1 protein by inducing the endoplasmic reticulum stress response and upregulating BH3-only protein NOXA. *J Biol Chem*; 286(28):24882-24895

[3] Alberts B., Johnson A., Lewis J., Raff M., Roberts K. and Walter P. (2008) *Molecular Biology of the Cell*. 5th ed. New York. Garland Science; 1205-1256

[4] American Cancer Society. *Breast Cancer Facts and Figures 2011-2012*. Atlanta, GA: American Cancer Society; 21-23

[5] American Joint Committee on Cancer. Breast. In: *AJCC Cancer Staging Manual*, 7th ed. New York: Springer; 2010: 347-369

[6] Antoniou A., Pharoah P.D., Narod S., et al. (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*; 72(5): 1117-1130

[7] Bai L, Zhu WG (2006) p53: structure, function and therapeutic applications. *J Cancer Mol*; 2(4):141-153

[8] Burstein H.J., Prestrud A.A., Seidenfeld J., et al. (2010) American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol*; 28(23):3784-3796

[9] Cheang M.C., Chia S.K., Voduc D., et al. (2009) Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst*; 101(10):736-750

[10] Chène P. (2001) p53 as a drug target in cancer therapy. *Expert Opin Ther Patents*; 11(6): 923-935

[11] Collaborative Group on Hormonal Factors in Breast Cancer (2012) Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol*; 13(11): 1141-1151

[12] De Bruin E.C. and Medema J.P. (2008) Apoptosis and non-apoptotic deaths in cancer development and treatment response. *Canc Treat Rev*; 34:737-739

[13] Devarajan E., Sahin A.A., Chen J.S., Krishnamurthy R.R., Aggarwal N., Brun A.M., Sapino A., Zhang F., Sharma D., Yang X.H., Tora A.D. and Mehta K. (2002) Down-

regulation of caspase 3 in breast cancer: a possible mechanism for chemo-resistance. *Oncogene*; 21(57):8843-8851

[14] Danial N.N. and Korsmeyer S.J. (2004) Cell death: critical control points. *Cell*; 116(2): 205-219

[15] Gao S. and Scott R.E. (2003) Stable overexpression of specific segments of the P2P-R protein in human MCF-7 cells promotes camptothecin-induced apoptosis. *J Cell Physiol*; 197:445-452

[16] Gasco M., Shami S., Crook T. (2002) The p53 pathway in breast cancer. *Breast Cancer Res*; 4:70-76

[17] Gerl R. and Vaux D.L. (2005) Apoptosis in the development and treatment of cancer. *Carcinogenesis*; 26(2):263-270

[18] Ghobrial I.M., Witzig T.E. and Adjei A.A. (2005) Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin*; 55:178-194

[19] Goolsby C., Paniagua M., Tallman M. and Gartenhaus R.B. (2005) Bcl-2 regulatory pathway is functional in chronic lymphocytic leukaemia. *Cytometry B Clin Cytom*; 63(1):36-46

[20] Gradishar W.J. and Wood W.C. (2008) Advances in Breast Cancer Management. In: Rosen S.T. ed. *Cancer Treatment and Research*. 2nd ed. New York, USA. Springer Science & Business Media, LCC; 199-149

[21] Green D.R. and Walczak H. (2013) Apoptosis Therapy: Driving Cancers down the road to ruin. *Natur Med*; 19:131-133

[22] Gross A., McDonnell J.M. and Korsmeyer S.J. (1990) BCL-2 family members and the mitochondria in apoptosis. *Genes Dev*; 13:1899-1911

[23] Hunt K.K., Robb G.L., Storm E.A. and Ueno N.T. (2008) Breast Cancer. In: Buzdar A.U. and Freedman R.S. eds. *M.D. Anderson Cancer Care Series*. 2nd ed. New York, USA. Springer Science & Business Media, LCC; 47-51

[24] Kang M.H. and Reynolds C.P. (2009) Bcl-2 inhibitors: Targeting mitochondrial apoptotic pathways in cancer therapy. *Clin Cancer Res*; 2009, 15:1126-1132

[25] Kasibhatla S. and Tseng B. (2003) Why target apoptosis in cancer treatments? *Mol Canc Therap*; 2:573-580

[26] Kelsey J.L., Gammon M.D. and John E.M. (1993) Reproductive factors and breast cancer. *Epidemiol Rev*; 15(1):36-47

[27] Kerr J.F.R., Wyllie A.H. and Currie A.R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*; 26:239-257

[28] Kuball J., Schuler M., Antunes Ferreira E., Herr W., Neumann M., Obenauer-Kutner L., Westreich L., Huber C., Wölfel T. and Theobald M. (2002) Generating p53-specific

cytotoxic T lymphocytes by recombinant adenoviral vector based vaccination in mice, but not man. *Gene Ther*; 9(13):833-843

[29] Kushi L.H., Doyle C., McCullough M., et al. (2012) American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *CA Cancer J Clin*; 62(1):30-67

[30] Lain S., Hollick J.J., Campbell J., Staples O.D., et al. (2008) Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. *Cancer Cell*; 13(5):454-463

[31] Lambe M., Hsieh C., Trichopoulos D., Ekbom A., Pavia M. and Adami H.O. (1994) Transient increase in the risk of breast cancer after giving birth. *N Engl J Med*; 331(1): 5-9

[32] Lavrik I.N., Golks A. and Krammer P.H. (2005) Caspases: pharmacological manipulation of cell death. *J Clin Invest*; 115:2665-2672

[33] Li G., Bush J.A. and Ho V.C. (2000) p53-dependent apoptosis in melanoma cells after treatment with camptothecin. *J Invest Dermatol*; 115:514-519

[34] Manjo G. and Joris I. (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol*; 146:3-15

[35] Miquel C., Borrini F., Grandjouan S., Aupérin A., Viguier J., Velasco V., Duvillard P., Praz F. and Sabourin J.C. (2005) Role of bax mutations in apoptosis in colorectal cancers with microsatellite instability. *Am J Clin Pathol*; 23(4):562-570

[36] Moela P., Choene M.S. and Motadi L.R. (2014) Silencing RBBP6 (retinoblastoma binding protein 6) sensitises breast cancer cells MCF-7 to staurosporine and camptothecin-induced cell death. *Immunobiology*; 219:513-601

[37] Morton J.P., Timpson P., Karim S.A., Ridgway R.A., Athineos D., Doyle B., Jamieson N.B., Oien K.A., Lowy A.M., Brunton V.G., Frame M.C., Jeffry Evans T.R. and Sansom O.J. (2010) Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. *PNAS*; 107(1):246-251

[38] Motadi L.R., Bhoola K.D. and Dlamini Z. (2011) Expression and function of retinoblastoma binding protein 6 (RBBP6) in human lung cancer. *Immunobiology*; 216:1065-1073

[39] National Comprehensive Cancer Network. (2013) NCCN Guidelines for patients: Breast cancer. *Version 3, 2013. Accessed at www.nccn.org on September, 11, 2014*

[40] Ocker M., Neureiter D., Lueders M. Zopf S., Ganslmayer M., Hahn E.G., Herold C. and Schuppan D. (2005) Variants of bcl-2 specific siRNA for silencing antiapoptotic bcl-2 in pancreatic cancer. *Gut*; 54(9):1298-1308

[41] Oltersdorf T., Elmore S.W., Shoemaker A.R., Armstrong R.C., *et al*. (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*; 435(7042): 677-681

[42] O'Brien M.A. and Kirby R. (2008) Apoptosis: a review of pro-apoptotic and anti-apoptotic pathways and dysregulation in disease. *J Vet Emerg Crit Care*; 18(6):572-585

[43] Pepper C., Hoy T. and Bentley D.P. (1997) Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. *Br J Cancer*; 76(7):935-938

[44] Pugh D.J., Ab E., Faro A., Lutya P.T., Hoffman E. and Rees D.J. (2006) DWNN, a novel ubiquitin-like domain, implicates RBBP6 in mRNA processing and ubiquitin-like pathways. *BMC Struct Biol*; 6, 1:5-7

[45] Raffo A.J., Perlman H., Chen M.W., Day M.L., Streitman J.S. and Buttyan R. (1995) Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. *Cancer Res*; 55(19):4438-4445

[46] Reis-Filho J.S. and Pusztai L. (2011) Gene expression profiling in breast cancer: classification, prognostication, and prediction. *Lancet*; 378(9805):1812-1823

[47] Rodrigues N.R., Rowan A., Smith M.E., Kerr I.B., Bodmer W.F., Gannon J.V., Lane D.P. (1990) p53 mutations in colorectal cancers. *Proc Natl Acad Sci USA*; 87(19): 7555-7559

[48] Roth J.A., Nguyen D., Lawrence D.D., et al. (1996) Retrovirus-mediated wild-type p53 gene transfer to tumours of patients with lung cancer. *Natur Med*; 2(9):985-991

[49] Sakai Y., Saijo M., Coelho K., Kishino T., Niikawa N. and Taya Y. (1995) cDNA sequence and chromosome localisation of a novel protein, RBQ-1 (RBBP6) that binds to the retinoblastoma gene product. *Genomic*; 30:98-101

[50] Schneider P. and Tschopp J. (2000) Apoptosis induced by death receptors. *Pharm Acta Helv*; 74:281-286

[51] Shangary S., Qin D., McEachern D., et al. (2008) Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumours and leads to complete tumor growth inhibition. *Proc Natl Acad Sci USA*; 105(10):3933-3938

[52] Shangary S. and Wang S. (2008) Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel approach for cancer therapy. *Annu Rev Pharmacol Toxicol*; 49:223-241

[53] Shen X.G., Wang C., Li Y., Wang L., Zhou B., Xu B., Jiang X., Zhou Z.G., Sun X.F. (2010) Down-regulation of caspase-9 is a frequent event in patients with stage II colorectal cancer and correlates with poor clinical outcome. *Colorectal Dis*; 12(12): 1213-1218

[54] Simons A., Melamed-Bessudo C., Wolkowicz R., Sperling J., Sperling R., Eisenbach L. and Rotter V. (1997) PACT: cloning and characterization of a cellular p53 binding protein that interacts with Rb. *Oncogene*; 14:145-55

[55] Suzuki K. and Matusubara H. (2011) Recent advances in p53 research and cancer treatment. *J Biomed Biotech*; 2011:1-4

[56] Turnbull C. and Rahman N. (2008) Genetic predisposition to breast cancer: past, present, and future. *Annu Rev Genomics Hum Genet*; 9:321-345

[57] Walker K., Bratton D.J. and Frost C. (2011) Premenopausal endogenous oestrogen levels and breast cancer risk: a meta-analysis. *Br J Canc*; 105(9):1451-1457

[58] Witte M.M. and Scott R.E. (1997) The proliferation potential protein-related (P2P-R) gene with domains encoding heterogeneous nuclear ribonucleoprotein association and Rb1 binding shows repressed expression during terminal differentiation. *Proc. Natl. Acad. Sci*; 94:1212-1217

[59] Wong R.S. (2011) Apoptosis in cancer: from pathogenesis to treatment. *Wong J Exper Clin Canc Therap*; 30(83):1-14

[60] Wu X., Liu X., Sengupta J., Bu Y., Yi F., Wang C., Shi Y., Zhu Y., Jiao Q. and Song F. (2011) Silencing of Bmi-1 gene by RNA interference enhances sensitivity to doxorubicin in breast cancer cells. *Ind J Exp Biol*; 49(2):105-112

IntechOpen