MINI-REVIEW

Recent Candidate Molecular Markers: Vitamin D Signaling and Apoptosis Specific Regulator of p53 (ASPP) in Breast Cancer

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Abstract

   Regardless of advances in treatment modalities with the invention of newer therapies, breast cancer remains a major health problem with respect to its diagnosis, treatment and management. This female malignancy with its tremendous heterogeneous nature is linked to high incidence and mortality rates, especially in developing region of the world. It is the malignancy composed of distinct biological subtypes with diverse clinical, pathological, molecular and genetic features as well as different therapeutic responsiveness and outcomes. This inconsistency can be partially overcome by finding novel molecular markers with biological significance. In recent years, newer technologies help us to indentify distinct biomarkers and increase our understanding of the molecular basis of breast cancer. However, certain issues need to be resolved that limit the application of gene expression profiling to current clinical practice. Despite the complex nature of gene expression patterns of cDNAs in microarrays, there are some innovative regulatory molecules and functional pathways that allow us to predict breast cancer behavior in the clinic and provide new targets for breast cancer treatment. This review describes the landscape of different molecular markers with particular spotlight on vitamin D signaling pathway and apoptotic specific protein of p53 (ASPP) family members in breast cancer.

Keywords: Breast cancer - molecular biomarkers - Vitamin D signaling pathway - apoptotic specific protein of p53

Breast Cancer: A Major Health Hazard

   Breast cancer is the most frequently diagnosed cancer occurring in females with an estimated burden of 1.38 million (23%) new cases worldwide, in 2008 (692,200 and 691,300 cases in developed and developing countries, respectively) (Ferlay et al., 2010) and ranks second most common cancer overall. About 458,400 deaths were projected because of breast cancer from which 60% of the deaths were reported in developing countries (Ferlay et al., 2010; Jemal et al., 2011). During past few years the incidence of breast cancer has risen in developed countries; in contrast the death rate has steadily decreased. However, in developing countries like India and others; both incidence and mortality rates have been increased (Jemal et al., 2011). In India, 115,251 new breast cancer cases with an age standardized incidence rate of 22.9 per 100,000 were estimated in 2008 (Ferlay et al., 2010) and by 2015; the incidence rate will reach just under 250,000 per year (Parkin et al., 2005).

   Like other malignancies, breast cancer is considered to be a genetic disease. Both genetic and non-genetic factors play a crucial role at various stages in tumorigenesis like initiation, development, progression and metastasis of breast cancer, which are mainly caused due to over expression and/or under expression, polymorphisms, mutation and/or deletion of specific genes or group of genes (Ventura & Merajver, 2008). One of the most important properties of breast cancer is its extreme heterogeneity, which is well recognized and clinically relevant but still poorly understood (Simpson et al., 2011). This unique feature of the malignancy provides characteristics like distinct pathological types, which differ in terms of clinical outcome and therapeutic response. Parker et al. (2009) have developed new intrinsic subtypes like Luminal A, Luminal B, Her 2-enriched and Basal like group by using advanced molecular techniques (microarray and qRT-PCR). Thus, growing knowledge of breast cancer cell molecular biology provides newer biomarkers in prediction of breast cancer behavior and contributes in the development of new strategies.

Current Scenario of Molecular Biomarkers in Breast Cancer Behavior

   The complexity of natural history of breast cancer set

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hurdles to clinicians in disease treatment and management, where molecular biomarkers act as a "tiebrakers" for selected breast cancer cases. Many molecular markers have been discovered, although only few of them i.e. CA-125, hormone receptors (ER- estrogen receptor and PR- progesterone receptor), human epidermal growth factor receptor 2 (Her 2/neu), and BRCA1 and BRCA2 (as risk factor in familial breast cancer) have been used routinely (Ventura and Merajver, 2008). Nowadays, gene expression profiling is the approach to predict treatment outcome and recurrence. It is available as prognostic tests commercially which includes Mamma Print (the 70 gene signature), Oncotype DX (the 21 gene recurrence score assay), Theros H/I (HOXB13: IL17BR ratio) and Theros breast cancer index (combination of Theros H/I and a molecular grade index which include 5 genes). These tests are tremendously promising for predicting response to chemotherapy and/ or hormonal therapy. But they are not yet adopted in routine clinical assessment

due to certain issues which comprises of expenditure, validation, reproducibility, reporting and interpretation of results (Stadler and Come, 2009; Simpson et al., 2011). Consequently, there is a need for more cost-effective, technically simple and readily available methods. In spite of gene expression assays, there have been several number of *invivo* and *invitro* studies describing molecular markers in breast cancer from the past decades and in recent times.

Reports from Our Laboratory in Breast Cancer Research

Previous reports from our laboratory have discussed imperative biomarkers of breast biology to resolve their ability in diagnosis, prognosis, treatment monitoring and therapeutic targets (Patel et al., 1990a: 1990b: 1996: 1998; Raval et al., 1997: 2004; Bala et al., 2001:2003; Shah et al., 2008: 2009a: 2009b) As documented in the Table 1, clinical significance of different biomolecules like gelatinases mainly gelatinase A i.e. Matrix metallo proteinase 2 (MMP-2) and gelatinase B i.e. Matrix metallo

Table 1. Summary of Studied Biomarkers from Our Laboratory

Biomarkers		Inferences	Ref
Invasion and metastasis	MMP-2 and MMP-9	Higher expression of active forms of MMP-2 and MMP-9 and their significant association with lymph node involvement in breast cancer were observed.	Shah et al., 2009b
Glycosylation	Seromucoid fraction	Seromucoid fraction levels can be used to distinguish between breast carcinoma pateints and healthy participants. A strong linkage was detected between favorable treatment response and decline in serum markers (Hexoses and mucoid proteins) levels.	Patel et al., 1990b: 1998
	Fucose	Serum fucose level exhibited significant higher value in breast cancer patients compared with controls.	Patel et al., 1990a
	Sialic acids	Different forms of sialic acid revealed considerably increased levels in breast cancer and may be used in diagnosis, prognosis and therapeutic monitoring. Serum LSA level can be used to differentiate infiltrative ductal carcinoma from lobular carcinoma.	Patel et al.,1990a,b; Raval et al., 1997:2004
	Sialyltransferase	Significant elevation in level of sialyltrasferase in breast cancer was correlated positively with presence of malignant tumor and negatively with response to treatment.	Raval et al., 2004
	Sialoproteins	More number of proteins with terminal α 2,6 sialic acid residues were examined in breast cancer patients than in controls and pathological controls.	Raval et al., 2004
	Glycoproteins	Alterations in all region glycoproteins (alpha, beta and gamma) were found in breast cancer patients at the time of diagnosis, during and after anticancer treatment.	Patel et al., 1996
Non-enzymatic antioxidants	Vitamins (A, E and C)	Studies confirms protective role of dietary intake of antioxidant vitamins. Importantly, considerable inverse association of vitamin E with disease status and treatment outcome is more striking.	Bala et al., 2001: 2003; Shah et al., 2009a
	β carotene	Reduced risk was established between elevated plasma β carotene level and breast cancer.	Bala et al., 2001: 2003; Shah et al., 2009a
Lipids	TC, HDL, LDL, VLDL and TG	Changes in circulatory lipid components may help to predict threat of breast cancer.	Bala et al., 2001: 2003; Shah et al., 2008
Others	Alkaline DNase and heat stable alkaline phosphatase	Their levels showed significant differences between breast cancer patients and controls.	Patel et al., 1990a, Raval et al., 1997

proteinase 9 (MMP-9), different forms of sialic acid i.e. lipid associated sialic acid (LSA), total sialic acid, free sialic acid and protein bound sialic acid, sialyltrasferase, glycoproteins, seromucoid fraction, non-enzymatic antioxidants, different lipids etc have been examined in breast cancer. As documented, various bio-molecular markers have significant role in clinic for breast cancer.

p53 - Apoptotic Specific Protein of p53 - Vitamin D Receptor Interactions: A Newly Evolved Era of Breast Cancer Research

To resolve heterogeneity of breast disease, it is essential to identify and characterize the molecular signatures and their clinical significance that may facilitate better understanding of the disease biology. Newer functional pathways and regulation barriers of biological significant proteins not only allow us to appraise prognosis of the disease, but also provide new therapeutic strategies.

It is very well documented that mutation rate of p53 in breast cancer is only 30% (Trigiante & Lu, 2006). Then why intact and functional p53 is unable to perform its role in breast tumors possessing wild type p53? Notably, newly discovered apoptosis specific regulator of p53 (ASPP) family members fulfill this uncertainty as they distinctively control wild type p53 induced apoptosis- one of the hallmark of cancer. Chromatin immunoprecipitation assays have shown that ASPP1 and ASPP2 selectively enhance the DNA binding activity of p53 in vivo on Bax and PIG3 promoters but not CDKN1A promoter (Trigiante and Lu, 2006). In addition to this, the current review also reflects a number of recent reports focused on p53-VDR interactions. The $1\alpha\ 25\ (OH)_2D_3$- VDR complex may play a role in maintaining genomic integrity and facilitating DNA repair. In which it may appears close cooperation between VDR action and p53 tumor suppressor pathway. VDR gene promoter contains p53 response elements. These raises the possibilities that the VDR and p53 co-operate to control cell differentiation, signal transduction and program cell death by several molecules. In support of this idea two critical p53 target genes, GADD45 and p21 are also known to be VDR target genes. Multiple p53 and VDR response element has been found in p21 promoter, suggesting this gene may be a common target for both pathways. The precise links between the p53 and VDR pathways suggested that mutant p53 can interact with VDR to control expression of

VDR target genes. Importantly, they showed that mutant p53 convert $1\alpha\ 25(OH)_2D_3$ into an antiapoptotic agent. In depth $1\alpha\ 25(OH)_2D_3$ analogous in combination with arsenic trioxide induces apoptosis in the p53 null HL-60 cell lines by downregulation of Bcl-2 and Bax. Therefore it is necessary to investigate all the potential mechanisms by which p53 and VDR interacts with each other (Maguire & Campbell, 2010).

Based on this idea, this review is predominantly focused on the recently revealed emerging area of molecular biomarkers (VDR and members of ASPP family) in breast cancer to improve the clinical patient management and its utility in clinical practice.

Mechanisms of Action of Vitamin D

Well-known classical endocrine functions of vitamin D for calcium homeostasis and bone metabolism are reviewed extensively. Recently, a growing body of evidence suggests the protective mechanism of vitamin D against breast cancer by autocrine/paracrine manner and many modestly reduced risk of breast cancer. In the first step 7-dehydrocholesterol is converted into vitamin D_3 in the skin after exposures to UV radiation. Vitamin D_3 is hydroxylated into 25 hydroxyvitamin D [25(OH) D] in the liver. Subsequent hydroxylation of 25(OH)D to $1\alpha\ 25(OH)_2D_3$ (calcitriol) occurs in the nephron, breast and other targeted tissues by the 1α hydroxylase enzyme (CYP27B1) (Bertone-Johnson, 2009). In autocrine mechanism breast epithelium also produced $1\alpha\ 25(OH)_2D_3$ from the circulatory 25(OH)D and it is the biologically active metabolite which is relatively small, lipophilic molecule that can easily penetrates by simple cell diffusion in the cell membrane and binds to the vitamin D receptor (VDR). Further, VDR heterodimerization with retinoid X receptor (RXR) takes place. The activated $1\alpha\ 25(OH)_2D_3$ - VDR - RXR complex specifically binds to vitamin D response elements (VDREs) and induces several gene expression (Figure 1) (Deeb et al., 2007). Degradation of unneeded $1\alpha\ 25(OH)_2D_3$ is accomplished by the enzyme CYP24A1 (24 Hydroxylase) for regulation of $1\alpha\ 25(OH)_2D_3$ synthesis.

The VDR Gene

A highly conserved VDR was discovered in 1969 for $1\alpha\ 25\ (OH)_2D_3$ (Slattery, 2007) and it is found widely throughout metazoans, even in certain non classified
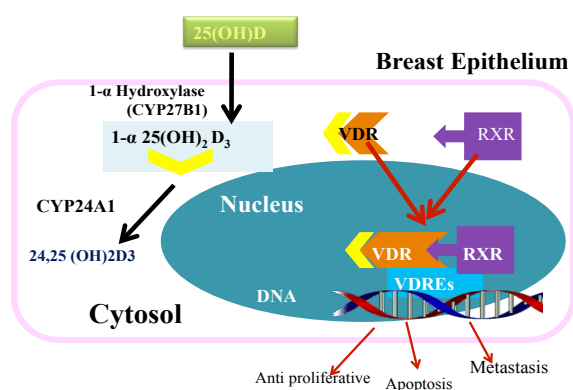

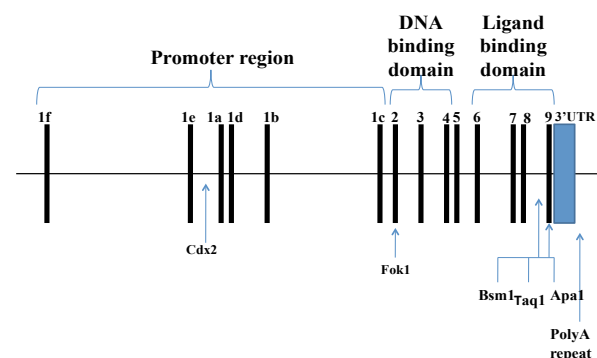**Figure 1. Vitamin D Signaling Pathway in Autocrine Manner**

Figure 2. Structure of Vitamin D Receptor (VDR) Gene

chordates such as lamprey (Thorne & Campbell, 2011). Since then, the role of VDR in the endocrine system and its presence and function in over 30 tissues and organs has been examined (Slattery, 2007). VDR is a nuclear transcription regulating factor and it belongs to the steroid hormone superfamily of receptors. It is located on chromosome 12q13 and spans over 100 kb. Currently, using gene sequencing and advent of the International Hap Map Project, the interesting findings of gene's structure, linkage distribution (LD) pattern and functional consequences of certain polymorphism has been increased (Rukin & Strange, 2007). Hence, VDR is a good candidate gene to study in the context of susceptibility. Moreover it is composed of six promoter and regulatory regions, untranslated exon 1a-1f and eight protein coding exon 2 to 9 in which (i) Exon 2 to 4 is encoded by DNA binding domain of the VDR peptide and it is responsible for interaction with VDREs in targeted genes. (ii) Exon 6 to 9 is encoded by the ligand binding domain and it is responsible for $1\alpha\ 25\ (OH)_2\ D_3$ binding (Figure 2) (McCullough et al., 2009).

Anticancer Effects of VDR and $1\alpha\ 25(OH)_2D_3$

a) Cell Cycle Regulation and Apoptosis: Direct regulations of cell cycle have been demonstrated by vitamin D metabolites, $1\alpha\ 25\ (OH)_2D_3$ and VDR in many cell systems. The most commonly reported effect has been observed due to an arrest at G0/G1 to S transition of cell cycle through multiple mechanisms (Samuel & Sitrin, 2008). Several invitro studies has shown that $1\alpha\ 25(OH)_2D_3$ inhibits the growth of human breast cancer cells. Especially, ER positive breast cancer cell lines appears to be more sensitive to the growth inhibitory effects compare to ER negative cell lines. In other malignancies, $1\alpha\ 25(OH)_2D_3$ also plays a growth inhibitory role by upregualting cell cycle inhibitors like p21, p27 and by downregulating cyclin A and cyclin D and also by decreased activity of CDKs and dephosporylation of the pRb (Krishnan et al., 2010; Narvaez et al., 2001) (Figure 3). According to Verlinden et al. (1998) the MCF-7 breast cancer cell line shows rapidly decreased cyclin D1 transcription level after treatment with $1\alpha\ 25(OH)_2D_3$. While protein levels only decreased after 72 hour of treatment. Also the transcription levels of p21 and p27 were up-regulated by sequential consistent changes in cell cycle distribution. A mechanism for down regulation of cyclin D1 and up-regulation of p21 and p27 is yet unknown. Numerous studies suggest that the $1\alpha\ 25(OH)_2D_3$ -VDR complex induces a program of genes which suppresses cell proliferation and induces differentiation in normal mammary gland. It may predict that expression of dysregulated VDR- mediated gene in mammary gland development or function are responsible for possibly predispose transformation of the cell (Welsh et al., 2003). However, indirect effect of $1\alpha\ 25(OH)_2D_3$ are observed on cell-cycle regulation by upregulation of Insulin Growth Factor Binding Protein 3 (IGFBP3) and transforming growth factor (TGFβ)–SMAD3 signaling cascades and by downregulation of the epidermal growth factor receptor (EGFR) signaling pathway (Deeb et al., 2007).

In addition to cell cycle regulation, $1\alpha\ 25(OH)_2D_3$ also plays key role in apoptosis by repressing the expression of the anti-apoptotic and pro-survival proteins like Bcl-2, Bcl-X_L or increasing the expression of pro-apoptotic proteins such as Bax, Bak and Bad. Based on this idea, several studies have reported that expression of Bcl-2 was down regulated by $1\alpha\ 25(OH)_2D_3$ in MCF-7 breast tumor and HL-60 leukemia cells. While, the expression of Bax and Bak were upregulated in several malignancies like prostate cancer, colorectal adenoma and carcinoma cells (Ylikomi et al., 2002). According to Wagner et al.(2003) induction of apoptosis was observed by $1\alpha\ 25(OH)_2D_3$ in Y79 retinoblastoma cells due to reciprocal changes between Bcl-2 and Bax protein (Figure 3). $1\alpha\ 25(OH)_2D_3$ also induced apoptosis through directly activate caspase effector molecules, although it is unclear whether $1, 25(OH)_2D_3$-induced apoptosis is caspase- dependent or independent (Deeb et al., 2007). It has also reported that some breast cancer cells shows potentiate TNF alpha induced apoptosis through the death receptor pathway, which is linked to the activation of caspases and phospholipase A2 (Colston and Hansen, 2002). A novel mechanism of $1\alpha\ 25(OH)_2D_3$-mediated apoptosis in epithelial ovarian cancer cells was proposed by Jiang et al.(2004), wherein they showed that $1\alpha\ 25(OH)_2D_3$ destabilizes telomerase reverse transcriptase (TERT) mRNA, therefore inducing apoptosis through telomere attrition resulting from the down-regulation of telomerase activity and it is first study which demonstrate stability of hTERT mRNA by a hormone. The proposed mechanism for induction of apoptosis followed by the $1\alpha\ 25(OH)_2D_3$ –VDR complex induces Vitamin D3– Upregulated Protein 1 and 2, which negatively regulates thioredoxin function and expression. Reduced levels of thioredoxin favor accumulation of reactive oxygen species (ROS), generating oxidative stress, as well as release and activation of apoptosis signal regulating kinase-1 (Welsh et al., 2003; Thorne & Campbell, 2011).

b) Anti Inflammatory Effect, Invasion and Metastasis: A variety of stimuli trigger chronic inflammation, which has been recognized as a risk factor for cancer development. Cancer related inflammation is characterized by presence of inflammatory cells at the tumor site and over expression of inflammatory mediators such as cytokines, chemokines, and prostaglandins in tumor tissues (Mantovani et al., 2008). $1\alpha\ 25(OH)_2D_3$ suppresses the expression of several
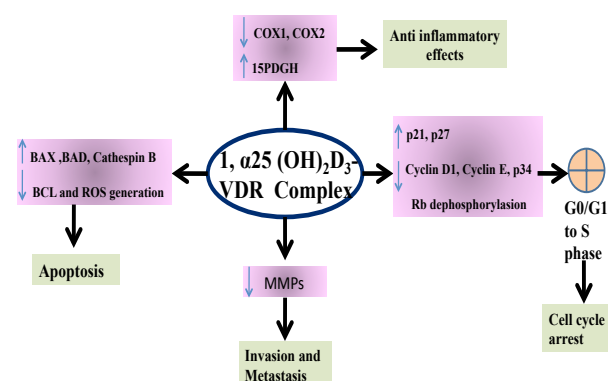

Figure 3. Role of Vitamin D in Apoptosis, Cell Cycle Regulation, Inflammation, Invasion and Metastasis

genes which are involved in prostaglandin pathway. Several *invitro* and *invivo* studies on breast cancer and prostate cancer showed $1\alpha\ 25(OH)_2D_3$ significantly decreases the expression of cyclooxygenase-2 (COX-2) and stimulates 15-PGDH levels (Krishnan & Feldman, 2011). However, several invivo studies showed the inverse correlation between VDR and both COX-2 and 15-Hydroxyprostaglandin Dehydrogenase (15-PGDH), as well as between PGE2 and $1\alpha\ 25(OH)_2D_3$ levels suggests a possible link between VDR associated target genes and prostaglandin metabolism in breast cancer and ovarian cancer (Figure 3) (Thill et al., 2010a; 2010b). Interestingly, a tight coupling between the expression of COX-2 and aromatase was observed in breast cancer patients (Brueggemeier et al., 1999; Brodie et al., 2001). $1\alpha\ 25(OH)_2D_3$ decreases the expression of aromatase in breast cancer cells which leads to decreases estrogen synthesis. There are two down regulatory mechanism of $1\alpha\ 25(OH)_2D_3$ on breast cancer through aromatase. (I) a direct repression of aromatase transcription via promoter II through the VDREs promoter and (II) an indirect effect due to the reduction in the levels and biological activity of PGE2, which is a major stimulator of aromatase transcription through promoter II in breast cancer. $1\alpha\ 25(OH)_2D_3$ also down regulates the ER α levels by direct transcription repression of ER α promoter and down regulate hormone (E2) and ERα receptor. Thus, significantly reduces the levels of estrogen in ER positive breast cancer cells (Krishnan et al., 2010).

In addition to antiproliferative, apoptotic and antiinflammatory effects, several epidemiological evidences suggest that $1\alpha\ 25\ (OH)_2D_3$ play vital role in invasion, metastasis and angiogenesis. Like ER – negative breast cancer cells are invasive in vitro and highly metastatic in vivo and $1\alpha\ 25(OH)_2D_3$ reduces the invasive potential of cancer cells (Krishnan et al., 2010). The RWPE2 prostate cancer cell lines shows reduced MMP-9 and MMP-2 activity with concomitant decrease in invasion (Tokar & Webber, 2005). It also suppresses urokinase type plasminogen activator and tissue type plasminogen activator and increases expression of PA inhibitor 1 and MMP inhibitors (Koli & Keski-Oja, 2000).

ASPPs: Arbiters of Cell Survival and Apoptosis

In humans, the ASPP family comprises three members: ASPP1, ASPP2 and inhibitory ASPP (iASPP). The proposal of the contribution of ASPP in human cancer first came in 1996 from the crystal structural analysis of the DNA binding domain of p53, C-terminal ankyrin repeats and SH3 domain of ASPP2. Gorina and Pavletich (1996) showed that p53 amino acids to which ASPP2 protein binds- 178His, 181Arg, 243Met and 247Arg are found to be mutated in the human cancer. Prominently, the six most frequently mutated p53 residues disrupt ASPP2 binding to p53, from which 248Arg and 273Arg are involved in binding to both DNA and ASPP2. This newly described family of p53 interacting protein identifies a precise mechanism by which it specifically stimulates the apoptotic function of p53. Samuels-Lev et al. (2001)

demonstrated specific effect of ASPP1 and ASPP2 on the apoptotic and transactivation functions of p53 for expression of proapoptotic targets such as Bax, PUMA and PIG3 but failed to affect the cell cycle arrest function of p53 under the same condition. Subsequent studies carried out by Bergamaschi et al. (2003; 2004), further reported that ASPP1 and ASPP2 can also bind p63 and p73 and function as common activators of p53 family members and iASPP inhibits p53 from triggering the apoptotic pathway. Accordingly, in response to cellular stress like DNA damage and oncogene activation, p53 family proteins are stabilized to direct a cell towards apoptosis. The binding of ASPP family proteins selectively modulate the apoptosis function of p53 family proteins and finally decide cell fortune between life and death (Figure 4). Furthermore, a mouse model study by Vives et al. (2006) and other invitro and invivo studies by Samuels-Lev et al. (2001), Bergamaschi et al. (2003) and Lettre et al. (2004) sustaining that ASPP1 and ASPP2 act as tumor suppressors, at the same time, iASPP as an oncogene.

The ASPP Family Genes

All the three members of ASPP family are encoded by three different genes that are located on three different human chromosomes- ASPP1 by PPP1R13B at 14q32.33, ASPP2 by TP53BP2 at 1q42.1 and iASPP by PPP1R13L at 19q13.32-3. These genes shares highly conserved sequence homology in carboxyl (C)-terminal part which contains ankyrin repeats, an SH3 domain and a prolin rich region. The amino (N)-terminus is only conserved in the ASPP1 and ASPP2. Figure 5 depicts structure of ASPP family genes in whichb, right side designate each ASPP members and its splice variants whereas, left side designate number of amino acids in length. Thus, the
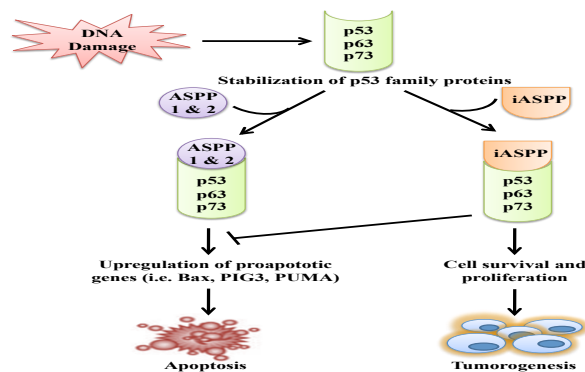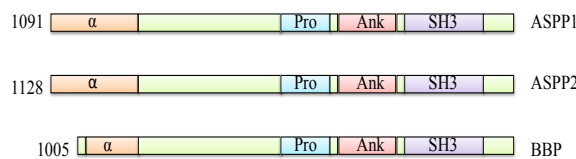

Figure 4. Functions of ASPP Family Proteins

Figure 5. Structures of Human ASPP Family Genes

nomenclature of the ASPP family is based on the domain organization of proteins (ankyrin repeat, SH3 and prolin rich domain containing protein) as well as their functions (apoptosis stimulating protein of p53).

ASPP2 is first recognized and well characterized member of ASPP family which encodes a protein of 1128 amino acids. It is a full protein form of previously identified p53 binding protein 2 (53BP2)/Bcl-2 binding protein (BBP), whereas ASPP1 is a novel protein among all members and contain 1091 amino acids (Samuels-Lev et al., 2001). Notably, iASPP is most phylogenetically conserved from worm to human by sharing 38% amino acid identity and 78% similarity in the ankyrin repeats and SH3 domian and only ASPP family member that identified in Caenorhabditis elegans- lower organism (Bergamaschi et al., 2003). Originally, iASPP was identified as a Rel A/p65 associated inhibitor (RAI) of 315 amino acids in length (Yang et al., 1999). Subsequent studies demonstrated full length of RAI protein, iASPP containing 828 amino acids in humans and in C. elegans its homologoue is named as Ce-iASPP containing 769 amino acids encoded by ape-1 gene (Bergamaschi et al., 2003; Slee et al., 2004).

ASPPs: Interaction with p53

The ASPP family members interact with p53 family members (p53, p63 and p73) via their C-terminus (ankyrin repeats and SH3 domain) (Robinson et al., 2008). This observation implies that iASPP compete with ASPP1 and ASPP2 to occupy p53 binding domain and result of this competition may provide another important level of regulation for the p53 response. Interestingly and importantly, ASPP family members also bind to the prolin rich region of p53 in addition to the DNA binding domain, which displays polymorphic loci at codon 72 in humans (Figure 6). Bergamaschi et al. (2006) described selective regulation of codon 72 variants by ASPP family members, particularly iASPP, bind to and control the activity of p53Pro72 more efficiently than that of p53Arg72,

A) Interaction between p53Pro72 and ASPP family members

B) Interaction between p53Arg72 and ASPP family members

Figure 6. Modulation of Function of p53 Codon 72 Isoforms

indicating that p53Arg72 activates apoptosis more capably than p53Pro72 due to getaway from negative regulation by iASPP. Hence, the most efficient way to inactivate the apoptotic function of p53Arg72 in human tumorigenesis is by intragenic mutation. In contrast, inactivation of the p53Pro72 isoform can occur by a reduction in the expression of ASPP1, ASPP2 or overexpression of iASPP, in addition to mutation in p53 itself. It suggests that consideration of ASPP family member expression and p53 polymorphic variants together can provide hint about cancer susceptibility, disease prognosis and new strategies to treat cancer.

The C-terminal fragment also mediate the interactions of ASPP proteins with several other biologically important proteins apart from p53, including RELA/p65 (subunit 3 of nuclear factor-kB), Bcl-2, adenomatous polyposis coli-like, Hepatitis-C core protein, amyloid-β-precursor protein-binding protein 1, YES-associated protein-1, protein phosphatase 1 (Trigiante & Lu, 2006). So far, most of the talk was focused on how the ASPP family proteins interact with p53 family but now it is also important to understand biological significance of these family protein interactions with other proteins which remains largely to determine.

ASPPs: Task in Breast Cancer

In the past decade, several studies confirm that ASPP1 and ASPP2 are coactivators of p53; whereas iASPP is a key inhibitor and they together selectively influence apoptosis. Recently reported studies have emphasized on deregulated expression of ASPP family proteins in a variety of human cancers. Initial study by Samuels-Lev et al. (2001) provides the first confirmation that ASPP1 and ASPP2 play a noteworthy role in tumor suppression by regulating p53 apoptosis function. They demonstrated frequently down regulation of ASPP1 and ASPP2 m-RNA expression in human breast tumor expressing wild type p53 but not mutant p53 and conclude that there is a selective advantage for tumor cells to lose the expression of ASPP1 and ASPP2 in human breast tumor showing wild type p53. Same group have reported over expression of iASPP in seven of eight human breast carcinoma possessing wild type p53 and normal levels of ASPP, suggesting a positive selection in human tumors retaining wild type p53. Considering both the study together it can be concluded that expression of ASPP family members are altered in almost 80% of the human breast carcinoma (Bergamaschi et al., 2003).

Another study showed low expression of ASPP1 and ASPP2 in breast cancer cell line (MCF-7) retaining wild type p53 with other two cell lines for hepatocellular carcinoma (HEPG-2) and lung cancer (A549) (Liu et al., 2005). Cohort study of 24,697 Danish postmenopausal women revealed a strong association between human chromosome 19 encoding iASPP region and breast cancer (Nexo et al., 2008). Liu et al. (2008) use RNA interference technology (RNAi) in order to investigate iASPP gene expression and apoptosis changes to provide a new strategy to resume cancer suppressing function of p53. After transfection, they observed decreased iASPP expression, while increase in apoptosis rate. Reduction in
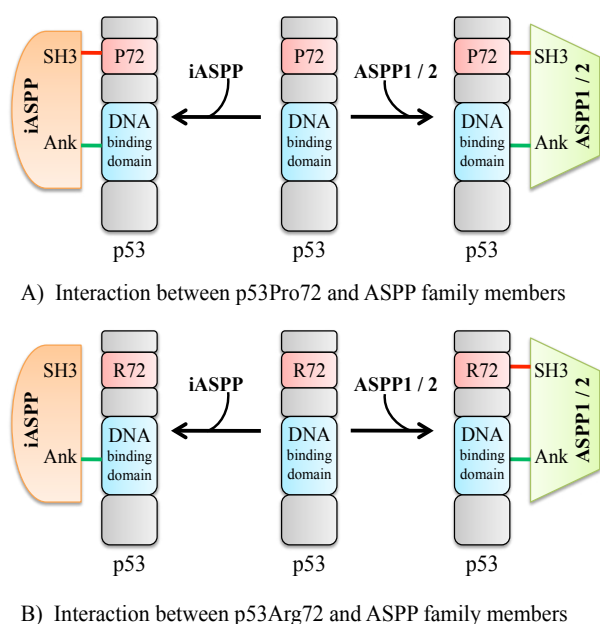
ASPP2 expression has been observed in microarray study of both invasive and metastatic breast cancer samples compared to normal breast samples, suggesting possible involvement of ASPP2 in breast cancer progression (Sgroi et al., 1999). Study carried out by Cobleigh et al. (2005) demonstrated independent association of ASPP2/ TP53BP2 gene with distal recurrence in breast cancer patients. They have linked higher ASPP2 expression with longer distal recurrence free survival. Another microarray study of RNA samples showed variation in TP53BP2 gene expression among BRCA1 or BRCA2 mutation carriers and sporadic breast cancer patients (Hedenfalk et al., 2001).

Reports for other malignancies like leukemia, hepatocellular carcinoma, lung cancer and prostate cancer (Mori et al., 2004; Trigiante and Lu, 2006; Chen et al., 2010; Zhao et al., 2010; Zhang et al., 2011) also show altered expression of ASPP family proteins in both cell lines and tissue. These findings signify down regulation of ASPP1 and ASPP2 and overexpression of iASPP may contribute in tumorogenesis, disease progression and may have potent therapeutic application. Importantly, inhibition of overexpression of iASPP may become a new strategy to resume the tumor suppressing function of p53. Moreover, gene knockdown of iASPP in different cancer cell lines (Liu et al., 2009; Chen et al., 2010; Li et al., 2011; Liu et al., 2011; Zhang et al., 2011) with mutant/ defective p53 or wild type p53 by using RNAi resulted into reduced mRNA and protein expression of iASPP and led to cell growth deceleration and induction of apoptosis, suggestive of additional functions of this oncoprotein in p53-independent manner. Furthermore, genetic polymorphisms at both TP53BP2 and iASPP have also been reported (Ju et al., 2005; Su et al., 2007) in gastric and non-small cell lung cancer respectively. Significant association was found between gastric cancer and different single nucleotide polymorphisms in TP53BP2 gene (g.206692C>T, g.198267A>T, g.164895G>A and g.152389A>T), whereas A allele of iASPP (A67T) was linked to treatment response to combined chemotherapy and radiotherapy in non-small lung cell carcinoma. Neither of ASPP1 or ASPP2 mutation has been identified in cancers till date. Conversely, Park et al. (2010) described a deletion mutation in the A7 repeats (c.576delA) of ASPP2 in high microsatellite instability (MSI-H) gastric and colorectal cancer but not in those with low microsatellite instability. Although frequency of this framshift mutation (p.Val193fsX1) is not high, but might possibly contribute to pathogenesis in MSI-H cancers.

Conclusion

We have reviewed the concepts of diverse biomarkers in breast cancer with highlights on newly evolved era of molecular markers in basic breast cancer research. In the era of targeted therapies, the combination of molecular factors into clinical approaches for prevention, prognosis, drug targets and treatment response appeal interesting findings. Newly discovered p53 interacting molecules and its up and down regulation together open a new route of breast cancer biology. Therefore, we have described the vitamin D functional pathways and the ASPP family to come across differences between breast cancer cells and healthy cells that may principally represent preventive strategy and rationally designed therapeutics. Frequently down expression of ASPP1 and ASPP2 or increased expression of iASPP offer to be defined as the mechanism involved in preventing wild type p53 and other p53 family proteins from working efficiently. Accordingly, the ASPP family member may provide prognostic markers and also allow us to develop new drug targets in combination with standard chemotherapy to produce additive or even synergistic effect. Inhibition of iASPP increase options in targeting the p53 family by restoring wild type p53 function or activate the p53 related protein p73. Whereas, vitamin D may play a protective role against mammary transformation and several important mechanisms are responsible for anti proliferative effects of vitamin D through different molecules which are involved in cell cycle regulation includes p21, p27, cyclin D1 and cyclin E. Vitamin D metabolites also induce apoptosis by affecting the levels of caspases, Bcl2, Bax and BAD regulatory proteins. So, vitamin D analogues, dietary vitamin D and high dose of 1α 25 $(OH)_2D_3$ combination with other compounds that are partially potent in regulating cell growth and differentiation can be use in anticancer therapeutics. Thus, ASPP expression pattern, p53 codon 72 and VDR gene polymorphisms and vitamin D metabolites all together may make available molecular findings of breast cancer susceptibility, prognosis and therapeutic strategies.

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