Review Article

Molecular basis of breast cancer

Layla J. Al-Mansouri, BSc, MSc, *Majed S. Alokail,* MSc, PhD.

ABSTRACT

Breast cancer is the most frequent cancer in women and represents the second leading cause of cancer death among women (after lung cancer). A common phenotypic abnormality of breast cancer cells is dysregulation of cell cycle control. The transformation of normal cell to a cancer cell appears to depend on mutation in genes that normally control cell cycle progression, thus leading to loss of the regulatory cell growth. We summarize here the molecular regulation of mammary carcinoma with regards to the most prominent oncogenes and tumor suppressor genes and their outcome in terms of cellular prognosis, and tumor development.

Saudi Med J 2006; Vol. 27 (1): 9-16

The mammalian cell cycle has been divided into a series of sequential phases. The G_1, S, G_2, and M phases are sequentially transition in response to growth factor or oncogenic stimulation. The S phase (DNA synthesis) and M phase (mitotic phases) are preceded by G_1 and G_2 (gap phases), and during the transition of the cell cycle, distinct checkpoints are inactivated.[1] These checkpoints provide mechanisms by which the intracellular compartment senses a favorable growth factor environment and continually transduces this information to ensure genetic integrity during cellular replication. The presence of DNA damage and the integrity of mitotic spindles are assessed during transition between these phases of the cell cycle. Cell cycle progression is orchestrated by the relative activity of a family of serine threonine kinases.[1]

Genome instability appears to be one of the earliest recognizable phenotypes and may be present even in histologically normal tissue. In fact, inherited cancer syndromes often involve this phenotype, for example, the breast-cancer susceptibility gene 1 and 2 (BRCA-1 and BRCA-2) breast/ovarian cancer risk genes are both involved in DNA repair.[2,3] Genome instability, whether inherited or not, results in a greater potential to develop genetic changes such as gene loss, gene amplification, point mutations and chromosomal translocations.[4,5] While most of these subsequent changes may result in cell death, some can affect key genes involved in cell survival, proliferation, invasiveness, motility, drug resistance and other malignant characteristics.[4,5] Cellular pathways affected by these genetic changes are highly interactive with each other. These complexities explain why diagnostic and therapeutic applications have progressed slowly.

Tumor genesis is thought to result from a series of progressive changes, including inactivation of tumor suppressor genes and activation of oncogenes. To determine the natural history of tumor and its response to chemotherapy, we should understand the vital role of cellular processes, which trigger, regulate and affect both programmed cell death (apoptosis) and cell proliferation.[2,3] Oncogenes and tumor suppressors

From the Department of Biochemistry, College of Science, King Saud University, Riyadh, *Kingdom of Saudi Arabia*.

Received 30th July 2005. Accepted for publication in final form 25th October 2005.

Address correspondence and reprint request to: Dr. Majed S. Alokail, Cancer Research, Oncology Unit, Somers Cancer Sciences Building, MP824, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, *United Kingdom*. Tel. +44 (23) 80796192 Fax. +44 (23) 80705152. E-mail: msalokail@yahoo.com

have opposite effects on the progression of cell cycle, so it was suggested that the growth abnormalities of tumor cells resulted from both too little of cell cycle breaks (tumor suppressors) and too little of the cell cycle accelerators (oncogenes).[6,7]

In mammary carcinomas, numerous factors related to and appear to influence the survival of patients with breast carcinoma. These molecular markers might merit reconsideration if individual genetic alterations can be targeted by means of specific therapies. These markers have been classified into 2 groups depending on there role in the development of cancer; oncogenes and tumor suppressor genes.[1,2,6] An oncogene results from a gain of function mutation of a proto-oncogene that generates a tumorigenic product, whereas mutation of a tumor suppressor causes a loss of function in the ability to restrain cell growth.[6] Many of the genes classified as oncogenes falls into categories of abnormally activated growth factors, growth factor receptors, intracellular signaling molecules and nuclear transcription factors. On the other hand, tumor suppressor genes mostly related and known to influence the cell cycle machinery are pRb and p53.[6] Moreover, there has been much attention focused on oncogenic components of the cell signaling system, such as the HER-2/Neu cascade.[8,9] In this article, we will discuss the most important oncogenes and their link to many end events, including cell proliferation, angiogenesis and apoptosis and their role in breast tumor progression.

The p53. P53 is regarded as a tumor suppressor gene and involved in distinct functions at the cellular level, as regulation of normal cell growth and division, gene transcription, DNA repair and genomic stability.[10] As a consequence, disruption of p53 function promotes checkpoint defects, cellular immortalization, genomic instability, and inappropriate survival allowing the continued proliferation and evolution of damaged cells.[11] In addition to its role in tumor suppression, p53-dependent apoptosis contributes to the chemotherapy induced cell death.[12] The protein product of the p53 gene (located on chromosome 17p13.1) is a phospho-protein comprising 393 residues with 4 highly conserved domains.[13] Mutation of p53 gene can occur in a number of ways including missense, nonsense, and frame shift mutations and the missense mutations that usually results in an increased half-life of the protein product and accumulation of the mutant p53 protein.[14,15]

P53 is found to promote or repress the expression of target genes in response to DNA damage. Amongst the key p53 target genes, in which altered expression is detected in many cancers including breast cancer, are mdm2 and p21.[7] Murine double minute 2 (MDM2) gene (MDM2) codes for oncoprotein which controls p53 biological activity and targets p53 for destruction.[16] P21 mediates the tumor-suppressing effect of p53 by inhibiting cyclin dependent kinase (Cdk) complex activity, thus blocking the transition from G_1 to S phase mediating p53-dependent growth arrest.[7] Several studies have suggested that p53 status is an important determinant of tumor responsiveness to anti-neoplastic agents, where p53 mutation is found to be the most common genetic abnormality in human cancer, and in breast cancer seen in up to 50% of primary carcinomas.[17] Loss of normal p53 function can potentially result in the relative resistance of breast cancers to chemotherapeutic agents due to the loss of the apoptotic properties of p53.[18] The independent prognostic power of p53 is suggested to be weak and it is best combined with other cellular and biological parameters as estrogen and progesterone receptor expression, Bcl-2, Bax and Her-2 for the proliferative properties of the tumor.[19,20]

P53 has been shown to affect apoptosis by regulating the expression of Bcl-2 and Bax which inhibit and promote apoptosis respectively. The reduced Bcl-2 and increased Bax expression observed in a p53-dependent manner following treatment with systemic chemotherapy suggest that analysis of the Bcl-2/Bax ratio as well as p53 status offers more practical information on tumor response following systemic chemotherapy.[21] The most intuitive link between p53-mediated transactivation and apoptosis comes from its ability to control transcription of proapoptotic members of the Bcl-2 family, including the multidomain member Bax.[22] The promoter of this gene harbor consensus p53 response element that is capable of binding p53 in vitro, resulting in the increasing the ratio of pro- to anti-apoptotic Bcl-2 proteins, thereby favoring the release of apoptogenic proteins from the mitochondria, caspase activation, and apoptosis.[23]

The BCL-2. The Bcl-2 family members can be both pro-survival (Bcl-2 and Bcl-XL) and pro-apoptotic (Bax, Bad and Bid). Bcl-2 and Bcl-XL have all 4 domains (BH1, BH2, BH3 and BH4), while Bax has only BH1, BH2 and BH3 (no BH4), and Bad and Bid are part of the BH3 sub-family, with only BH3 domain.[24] During the developmental period, Bcl-2 is expressed in every tissue, however in adults it is expressed in proliferating or reserve cells. In breast tissue, Bcl-2 is known to be expressed in the normal mammary epithelial cells of the non-pregnant female and during early pregnancy but undetectable in the lactating or involuting mammary gland.[25] A

balance between cell proliferation and apoptosis controls the development and function of normal breast, as a result the anti-apoptotic function of Bcl-2 is required in adult mammary glands, which also express estrogen receptor.[26] Bcl-2 protein is known to promote cell survival, but does not proliferate the cells, by prolonging cell cycle where Bcl-2 expressing breast cancer cells showed increased doubling time, decreased S phase fraction, and G_1/G_0 fraction, where these processes are subject to distinct genetic control.[26,27]

Bcl-2 gene (18q21.3) was initially identified at a breakpoint in a chromosomal translocation (t14:18) that occurs in human B-cell lymphomas.[28] The 26kDa oncoprotein Bcl-2 localizes to intracellular membranes including the endoplasmic reticulum, mitochondria and perinuclear membrane. It anchors to intracellular membranes through a hydrophobic region located near its C terminus, while the major portion of the molecule is in the cytoplasm.[29] It may directly bind to the mitochondrial channel and control the mitochondrial membrane permeability by stabilization.[30] Bcl-2 protein is well known to protect cells from various stresses such as ionizing radiation exposure, chemotherapeutic agent treatment and serum and growth factor withdrawal. It also facilitates recovery from DNA damage after oxidative stress.[31] An important correlation was found between Bcl-2 expression and ER and PR status, where 17ß-estradiol had an effect on the anti-apoptotic protein Bcl-2 resulting its upregulation but down regulated Bcl-X protein.[32] In addition tamoxifen, an antiestrogen, blocked the down regulation of Bcl-X by 17ß-estradiol. These findings suggests that Bcl-2 family proteins may be regulated through unique pathways modulated by estradiol, thus the expression of ER by the tumor cells controls the Bcl-2 expression or down regulation.[26,32]

In normal adult state cells, Bcl-2 makes heterodimer with a pro-apoptotic member of the Bcl-2 family, namely Bax coded by gene located on chromosome 19q13.3. While in pro-apoptotic conditions, intracytoplasmic Bax overexpression makes the equilibrium shift to the Bax homodimer formation which forms a channel in the mitochondrial membrane allowing the exit of cytochrome C which results in apoptosis.[33] In anti-apoptotic conditions the equilibrium shift to the Bcl-2 homodimer blocks the channel forming activity of Bax.[34] In addition, it has been shown that phosphorylation of Bcl-2 at serine residues leads to the loss of Bcl-2 anti-apoptotic function and anti cancer drugs induces Bcl-2 phosphorylation followed by apoptosis in prostate cancer cell lines. These results in decreased Bcl-2 binding to the proapoptotic Bax protein.[35] These results were suggested to lead to a novel role for Bcl-2 as a guardian of microtubules integrity which are important in chromosome segregation. Bcl-2 responds to damage in microtubules by leaving its normal anti-apoptotic function through its phosphorylation and functional inactivation, leading to induction of apoptotic death of cells exhibiting such alterations.[36]

The Bag-1. Bag-1 (Bcl-2-associated athanogene) is identified as Bcl-2 interacting anti-apoptotic protein, it also binds hormone receptors (as ER) and inhibits hormone induced apoptosis, this, beside other Bag-1 interactions, plays an essential role in the oncogenesis and disease progression of breast cancer, since Bag-1 interacts with Bcl-2 and is upregulated by mutant p53.[37] Bag family proteins were originally identified by their ability to interact with the anti-apoptotic Bcl-2 protein.[38] Six human Bag proteins have been reported and only 4 of them (Bag-1, -3, -4, and -6) have been confirmed in vivo and shown to interact with heat shock proteins (Hsc70/Hsp70).[39] The Bag-1 gene (9p12) encodes 4 isoforms of the Bag-1 proteins, expressed through alternative translation initiation sites, these are Bag-1L (52kDa), Bag-1M (46kDa), and Bag-1S (29kDa). Bag-1L is located primarily in the nucleus, while Bag-1M and Bag-1S proteins are found mainly in the cytoplasm. Bag-1M is occasionally found in the nucleus depending on the cell type and whether the cells were exposed to stress conditions.[40]

All Bag-1 isoforms contain a C-terminal "Bag domain" which plays a key role in mediating many Bag-1 functions. The C-terminal domain has 110-124 amino acids comprised of 3 anti-parallel α helix and each one have approximately 30-40 amino acids in length. The first and second helices interact with the serine/threonine kinase Raf-1, while the second and third helices bind with the ATPase domain of Hsc70/Hsp70 to stimulate the ATP hydrolysis of Hsc70 by accelerating the exchange of ADP/ATP.[41] The Bag domain contributes to the anti-apoptotic activity of Bag family proteins; it enhances the cell resistance to apoptotic stimuli. Bag-1 binds with a variety of intracellular proteins which regulates several processes relevant to cancer as cell division, cell survival and cell migration.[42] Bag-1 binds to serine/threonine kinase Raf-1, which is normally activated by Ras, then the activated Raf-1 turns on its downstream extracellular signal-related kinase (ERK) in stimulating cell proliferation.

Moreover, when cells are under stress, the increased Hsp70 may compete with Raf-1 for Bag-1 binding which inhibits the subsequent events as DNA

synthesis, resulting in cell cycle arrest.[43] Thus, Bag-1 is suggested to function as a molecular switch, that encourages cells to proliferate in normal conditions,[44] then becomes quiescent under a stressful environment. As a result of that, Bag-1 overexpression provides a potential mechanism by which tumors lacking oncogenic Ras mutations. Hence, Bag-1 activates Raf-1 independent of Ras, activation of mitogen activated protein kinase (MAPK) pathway leads to mediated proliferative and survival signals.[42]

The resulting suppression of apoptosis by Bag-1, might contribute to the ability of Bag-1 to promote metastatic spread through enhanced cell motility.[45] Several other proteins are found to interact with Bag-1 including nuclear hormone receptors (NHR) where Bag-1 potentiates the activity of ER mediating proliferative and survival responses to estrogens in hormone dependent breast cancers.[46] Besides the NHR, Bag-1 found to interact with the anti-apoptotic Bcl2 protein and some tyrosine kinase receptors such as hepatocyte growth factor (HGF) and platelet derived growth factor receptors (PDGFR).[42] A positive correlation was represented by a study between the expression of Bag-1 and p53, this supports the finding that the activity of the Bag-1 promoter was upregulated by mutant p53 and this regulation was dose dependent with the increasing concentrations of the mutant p53.[4]

Furthermore, a relatively high level of positive correlation exists between the expression of Bag-1, ER and PR. Bag-1 expression was elevated in 92% (66% in other study) of invasive breast cancers wherein most of which exhibited cytosolic isoform "rarely found in normal mammary epithelium".[47,48] While a small portion showed nuclear expression, it might be correlated to the tumor grade/differentiation where relatively high levels of nuclear Bag-1 expression was found in low grade tumors.[48] These findings beside the forced expression of Bag-1 isoforms in human breast cancer cells resulted in enhanced survival during growth factor deprivation and accelerated growth in vivo. These data suggest that Bag-1 may be a reliable molecular marker for the pathogenesis and progression of breast cancer serving an independent prognostic factor in the management of breast cancer.

P27 and Skp2. Pelargonium flower break virus protein (p27) and S-phase kinase-associated protein 2 (skp2) are negative regulators of the cell cycle are considered tumor suppressor genes in that a loss of their function can contribute to malignant behavior. First isolated in 1993, p27 belongs to a family of cyclin-dependent protein kinase inhibitors (CKIs) known as Cip/Kip, whose other members are p21 and p57.[49] CKIs slow the progression of the cell cycle; p27 is capable of binding to a number of unique cyclin/CDK complexes to attenuate their activity, typically directing the cell toward arrest in the G1 phase. It has been demonstrated that p27 has separate binding sites for cyclin and CDK2 and that binding to this complex results in conformational changes of the catalytic cleft of CDK2.[49] Many roles of p27 have been proposed, including functions in modulation of drug resistance, cell differentiation, and protection from inflammation.[50] Supporting the role of p27 as a tumor suppressor, decreased expression has been documented in a wide range of human cancer cell lines. However, mutations of p27 appear to be an uncommon events in malignancy, occurring in only 1% of tumors in one study.[51]

In breast cancer, diminished expression of p27 is associated with shorter overall survival and shorter time to progression, and it seems to be a stronger independent predictor of outcome than either p53 alterations or tumor grade.[52] Stepwise, loss of p27 expression may be an event that parallels the transition of a cell from the normal to premalignant to malignant phenotypes.[53] Some degree of the poor prognosis conferred by loss of p27 expression may be related in part to a role in modulating cell-cell adhesion, and thus, tendency for metastatic spread. In one series, analyzing tumors smaller than 1 cm in diameter, p27 underexpression was found to be a strong predictor of lymphatic spread.[54] Affording further support for the importance of p27 in tumor behavior is the finding that antiestrogen compounds result in increased inhibition of CDK activity.[55] The S-phase kinase-associated protein Skp2 is required for the ubiquitin-mediated degradation of p27 and has been shown to experimentally increase oncogenicity and resistance to antiestrogens in vitro.[56] Skp2 may also be preferentially overexpressed in ER and HER-2 breast cancer, a subset of breast cancer recently defined as the "basal phenotype" by gene profile analysis.

The HER-2. One of the most promising tumor associated marker is the HER2/neu.[57] HER2/neu is a proto-oncogene located on chromosome 17q11.2-q12, encodes a 185-kDa transmembrane glycoprotein, with intracellular tyrosine kinase activity that belongs to the epidermal growth factor receptor family.[58] HER2/neu is expressed in a variety of tissues where it plays fundamental roles in development, proliferation, and differentiation. Although no direct ligand has been found for HER2/neu, it can be activated by its overexpression or transactivated by various ligands

of EGF family.[59] The activation of HER2/neu by the formation of heterodimers with other ErbB receptors is well described and involves prolongation of the signaling by ErbB2 containing heterodimers.[60] Ras/MAPK and PI3K/Akt are 2 downstream pathways of ErbB2, which link ErbB2 to its biological functions.[61]

An amplification or over expression of tyrosine kinase receptor HER2/neu is found in approximately 20-40% of patients with breast cancer, as a result HER2/neu is overexpressed on the cell surface leading to increased oncogenesis.[58] There is evidence that over-expression of HER2 and p53 is involved in breast cancer progression, indicating the coexistence of HER2 over-expression and accumulation of p53 protein is a strong prognostic molecular marker in breast cancer.[62,63] Furthermore, receptor tyrosine kinases (RTKs) are involved in a broad spectrum of cell growth and differentiation events. Receptor tyrosine kinases are classified based on sequence homology and domain organization. Type I RTKs include the epidermal growth factor receptor (EGFR) and the human EGF receptor (ErbB1, HER1) homologues ErbB2 (Neu, HER2), ErbB3 (HER3), and ErbB4 (HER4) also named as c-erbB1–4.[61] Overexpression of several members of this receptor family, especially EGFR and HER2, is associated with a variety of solid tumor malignancies.

The ErbB receptor tyrosine kinases, in particular ErbB1 and ErbB2, have roles in human cancer development, thus making them attractive targets for cancer therapies.[64] ErbB2 overexpression, generally attributable to gene amplification which occurs in 25–30% of breast cancer and correlates with shorter time to relapse and lower overall survival.[65] It has been observed that targeting of overexpressed active ErbB2 results in efficient inhibition of breast cancer cell proliferation, which proceeds via inhibition of intracellular signaling pathways and directly targets various members of the cell cycle machinery.[66] Interestingly, expression of ErbB3 is seen in many tumors that express ErbB2, including breast,[67] and ErbB3 has impaired kinase activity and it needs a dimerization partner to become phosphorylated and acquire signaling potential.[68-70]

The estrogen receptors (ER). Other molecular marker, usually associated with breast cancer is the ER. Estrogens have essential functions in both female and male physiology, especially 17ß-estradiol (E2) which plays central role in the proliferation and differentiation of responsive cells, through changing the expression profile of target genes within responsive tissues.[71] 17ß-estradiol target tissues can be divided in to 2 groups, the classical and non-classical E2 target tissues. The classical targets are the uterus mammary gland, placenta, liver, central nervous system, cardiovascular system and bone; and these tissues have a high ERα content.[72] The non-classical target tissues include prostate, testis, ovary, pineal gland, thyroid gland, parathyroids, adrenals, pancreas, gallbladder, skin, urinary tract and erythroid tissues. In these tissues, expression of ERα is either very low or not measurable, whereas ERß is highly expressed.[73]

In the mammary glands, ER is found in both the epithelial cells (ductal and lobular) and stromal cells and even during embryogenesis, ER is expressed in both the ductal epithelium and stroma, not in the lobular epithelium. One of the most interesting aspects of ERs in the breast is their dual role in both proliferation and differentiation.[74] E2 binds to transcription factors belonging to the nuclear receptor superfamily, in order to mediate its effect, the estrogen receptors (ERα and ERß), these are proteins encoded by 8 exons of 2 genes on different chromosomes.[75]

Genes relevant to tumorigenesis and thought to be regulated either directly (ER DNA binding) or indirectly (via cooperative interactions with other transcription factors), by ER encode proteins involved in apoptosis (as Bcl-2), cellular invasiveness (as cathepsin D), and cellular proliferation such as v-myc myelocytomatosis viral oncogene homolog (Myc) and transforming growth factor-alpha (TGFα).[76] On one hand, the expression of ERß is much lower in tumors than in healthy glands and in relation to ERα, ERß expression is reported to decrease during carcinogenesis.[77] Additionally, overexpression of ERß mRNA was reported in tumors from patients who became resistant to chemotherapeutics such as tamoxifen, where tamoxifen-liganded ERα and ERß have been shown to have strikingly different effects on activation number 1, AP-1 (a transcription factor) gene regulation with tamoxifen-liganded ERα exerting antagonistic effects.[78] While tamoxifen-liganded ERß agonistically activates AP-1 target genes in all cell types tested, thus increased tumor expression of ERß could be associated with the development of tamoxifen resistance mediated through AP-1 target genes.[78]

ERα, on the other hand, is a well established prognostic marker in breast cancer pathogenesis, and it is thought to be the primary predictive factor for endocrine response in breast tumors.[79] In clinic, factors as ERα, PR, and nodal status used to predict response to endocrine therapy, patients with ERα+ show 53% objective response rate to endocrine therapy, and this can be split into 69% for ERα+ PR+

and 11% of ERα+ PR-.[80] In addition, most tumors expressing mutant p53 belonged to the high-grade, poorly differentiated malignancies, this agrees with the observation that expression of ER and mutant p53 is completely inverse in breast cancer cells.[81] These data suggested that the association of p53 mutation with cell cycle progression led to rapidly growing cells with low amount of ER accumulation.[81,82] ERα is capable of binding to MDM2 and the NH2 terminus of the p53 protein, thus protecting p53 from being deactivated by the MDM2 ligand independently.[83] It has been reported that ER-positive breast cancer cells have significantly higher (up to 30 folds) MDM2 mRNA levels than ER- negative ones, and this observed stimulatory effect of ERα on MDM2 was carried out via elevated p53 transcriptional activity.[84]

Furthermore, in ERα+/EGFR+ tumors, individual tumor cells express high levels of only ERα or EGFR, but never both.[85] The EGFR+ cells in these tumors are also associated with a higher growth rate than the ERα+/low EGFR cells.[86] Interestingly, ERα and EGFR expression in the same cell is observed in normal and benign breast specimens,[87] suggesting that the interaction between these 2 signaling pathways is altered in breast cancer cells.

In conclusion, there are more questions than answers at this time regarding the putative functional significance of many prominent markers expression by breast cancer cells. It will be important to address whether breast cancer cells form similar patterned networks and this area of research merits further exploration, with potential benefits for molecular diagnosis and therapeutic intervention strategies. We hope that our paper will encourage us and other investigators to examine breast tumors and other cancers for the putative biological significance of the molecular expression of these markers in cancers cells. Nonetheless, in order to evaluate more accurately the overall risk of breast tumorigenesis, novel genetic and phenotypic traits need to be identified.

References

1. Hilakivi-Clarke L, Wang C, Kalil M, Riggins R, Pestell RG. Nutritional modulation of the cell cycle and breast cancer. ***Endocrine-Related Cancer*** 2004; 11: 603-622.
2. Maser RS, DePinho RA. Connecting chromosomes, crisis and cancer. ***Science*** 2002; 297: 565-569.
3. Evan DI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. ***Nature*** 2001; 411: 342-348.
4. Waldman FM, DeVries S, Chew KL, Moore DH, Kerlikowske K, Ljung BM. Chromosomal alterations in ductal carcinomas in situ and their in situ recurrences. ***J Natl Cancer Inst*** 2000; 92: 313-320.
5. O'Connell P, Pekkel V, Fuqua SA, Osborne CK, Clark GM, Allred DC. Analysis of loss of heterozygosity in 399 premalignant breast lesions at 15 genetic loci. ***J Natl Cancer Inst*** 1998; 90: 697–703.
6. Eissa S, Labib R, Khalifa A, Swelam N, Khalil F, El-Shenawy AM. Regulators of apoptosis in human breast cancer. ***Clin Biochem*** 1999; 32: 321-326.
7. Osborne C, Wilson P, Tripathy D. Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications. ***Oncologist*** 2004; 9: 361-377.
8. Sledge GW, Miller KD. Exploiting the hallmarks of cancer, the future conquest of breast cancer. ***European J Cancer*** 2003; 39: 1668-1675.
9. Nahta R, Hortobagyi GN, Esteva FJ. Growth factor receptors in breast cancer: potential for therapeutic intervention. ***Oncologist*** 2003; 8: 5–17.
10. Levine AJ. P53, the cellular gatekeeper for growth and division. ***Cell*** 1997; 88: 323-331.
11. Hussain SP, Harris CC. Molecular epidemiology of human cancer; contribution of mutation spectra studies of tumor suppressor genes. ***Cancer Res*** 1998; 58: 4023-4037.
12. Jhonstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. ***Cell*** 2002; 108: 153-164.
13. Lane DP. Exploiting the p53 pathway for cancer diagnosis and therapy. ***Br J Cancer*** 1999; 80:1-5.
14. Lai H, Ma F, Trapido E, Meng L, Lai S. Spectrum of p53 tumor suppressor gene mutations and breast cancer survival. ***Breast Cancer Res Treat*** 2004; 83: 57-66.
15. Soengas MS, Capodieci P, Herman JG, Polsky D, Mora J, Esteller M et al. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. ***Nature*** 2001; 409: 331-333.
16. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. ***Nature*** 1997; 387: 296-299.
17. Clahsen PC, van de Velda CJ, Duval C, Pallud C, Mandard AM, Delobelle-Deroide A et al. P53 protein accumulation and response to adjuvant chemotherapy in pre-menopausal women with node negative early breast cancer. ***J Clin Oncol*** 1998; 16: 470-479.
18. Degeaorges A, de Roquancourt A, Extra JM, Espie M, Boursyn E, de Cremoux P, et al. Is p53 a protein that predicts the response to chemotherapy in node negative breast cancer?. ***Breast Cancer Res Treat*** 1998; 47: 47-55.
19. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, et al. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. ***Breast Cancer Res*** 2003; 6: 24-30.
20. Zheng W, Lu J, Zheng J, Hu F, Ni C. Variation of ER status between primary and metastatic breast cancer and relationship to p53 expression. ***Steroids*** 2001; 66: 905-910.
21. Kobayashi S, Iase H, Ito Y, Yamashita H, Iwata H, Yamashita T et al. Clinical significance of bcl-2 gene expression in human breast cancer tissues. ***Breast Cancer Res Treat*** 1997; 42:173-181.
22. Yu J, Wang Z, Kinzler KW, Vogelstein B, Zhang L. PUMA mediates the apoptotic response to p53 in colorectal cancer cells. ***Proc Natl Acad Sci USA*** 2003; 100: 1931-1936.
23. Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of bax in the apoptotic response to anticancer agents. ***Science*** 2000; 290: 989-992.

24. Adams MD, Raman P, Judd RL. Comparative effects of englitazone and glyburide on gluconeogenesis and glycolysis in the isolated perfused rat liver. ***Biochem Pharmacol*** 1998; 5: 1915-1920.
25. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. Bcl-2 protein is topographically restricted in tissues characterized by apoptotic cell death. ***Proc Natl Acad Sci*** 1991; 88: 6961-6965.
26. Park SH, Kim H, Song BJ. Down regulation of Bcl-2 expression in invasive ductal carcinomas is both estrogen and progesterone receptor dependent and associated with poor prognostic factors. ***Pathol Oncol Res*** 2002; 8: 26-30.
27. Kumar R, Vadlamudi RK, Adam L. Apoptosis in mammary gland and cancer. ***Endocr Relat Cancer*** 2000; 7: 257-269.
28. Korsmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. ***Blood*** 1992; 80: 879-886.
29. Shimizu S, Narita M, Tsujimoto Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel. ***Nature*** 1999; 399: 483-487.
30. Zhu L, Ling S, Yu XD, Venkatesh LK, Subramanian T, Chinnaduri G, et al. Modulation of mitochondrial Ca homeostasis by bcl-2. ***J Biol Chem*** 1999; 274: 33267-33273.
31. Lee YJ, Chen JC, Amoscato AA, Bennouna J, Spitz DR, Suntharalingam M et al. Protective role of bcl-2 in metabolic oxidative stress-induced cell death. ***J Cell Sci*** 2000; 114: 677-684.
32. Leung LK, Wang TT. Differential effects of chemotherapeutic agents on the bcl-2/bax apoptosis pathway in human breast cancer cell line MCF-7. ***Breast Cancer Res*** 1999; 55: 73-83.
33. Quinn DI, Henshall SM, Sutherland RL. Molecular markers of prostate cancer outcome. ***Eur J Cancer*** 2005; 41: 858-887.
34. Reed JC. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. ***Curr Opin Oncol*** 1995; 7: 541-546.
35. Simstein R, Burow M, Parker A, Weldon C, Beckman B. Apoptosis, chemoresistance, and breast cancer: insights from the MCF-7 cell model system. ***Exp Biol Med*** 2003; 228: 995-1003.
36. Halder S, Basu A, Corce CM. Bcl-2 is the guardian of microtubule integrity. ***Cancer Res*** 1997; 57: 229-233.
37. Tang SC, Beck J, Murphy S, Chernenko G, Robb D, Watson P, et al. BAG-1 expression correlates with bcl-2 p53 differentiation, estrogen and progesterone receptors in invasive breast cancer. ***Breast Cancer Res Treat*** 2004; 84: 203-213.
38. Lee J, Takahashi T, Yasuhara N, Inazawa J, Kmada S, Tsujimuto Y. BAG, a bcl-2 binding protein that synergizes with bcl-2 in preventing cell death. ***Oncogene*** 1999; 18: 6183-6190.
39. Doong H, Vrailas A, Khon EC. What is in the BAG? - a functional domain analysis of the BAG family proteins. ***Cancer Letters*** 2002; 188: 25-32.
40. Takayama S, Krajewska S, Kitada S, Zapata JM, Kochel K, Knee D et al. Expression and location of Hsp70/Hsc70 –binding anti-apoptotic protein BAG-1 and its variants in normal tissues and tumor cell lines. ***Cancer Res*** 1998; 58: 3116-3131.
41. Sondermann H, Scheufler C, Schneider C, Hohfeld J, Ulrich-Hartl F, Moarefi I. Structure of a BAG/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. ***Science*** 2001; 291: 1553-1557.
42. Cutress RI, Townsend PA, Brimmell M, Bateman AC, Hague A, Packham G. BAG-1 expression and function in human cancer. ***Br J Cancer*** 2002; 87: 834-839.
43. Townsend PA, Cutress RI, Sharp A, Brimmell M, Packham G. BAG-1 prevents stress-induced long-term growth inhibition in breast cancer cells via chaperone-dependent pathway. ***Cancer Res*** 2003; 63: 4150-4157.
44. Barnes JD, Arhel NJ, Lee SS, Sharp A, Al-Okail M, Packham G et al. Nuclear BAG-1 expression inhibits apoptosis in colorectal adenoma-derived epithelial cells. ***Apoptosis*** 2005; 10: 301-311.
45. Takaoka A, Adachi M, Okuda H, Sato S, Yawata A, Hinoda Y et al. Anti-cell death activity promotes pulmonary metastasis of melanoma cells. ***Oncogene*** 1997; 14: 2971-2977.
46. Cutress RI, Townsend PA, Sharp A, Maison A, Wood L, Lee R et al. The nuclear BAG-1 isoform, BAG-1L, enhances oestrogen-dependent transcription. ***Oncogene*** 2003; 22: 4973-4982.
47. Tang SC, Shaheta N, Chernenko G, Khalifa M, Wang X. Expression of BAG-1 in invasive breast carcinomas. ***J Clin Oncol*** 1999; 17: 1710-1719.
48. Turner BC, Krajwski S, Krajwski M, Takayama S, Gumbs AA, Carter D, et al. BAG-1: a novel biomarker predicting long term survival in early-stage breast cancer. ***J Clin Oncol*** 2001; 19: 992-1000.
49. Russo AA, Jeffrey PD, Patten AK, Massague J, Pavletich NP. Crystal structures of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A-Cdk 2 complex. ***Nature*** 1996; 382: 325-331.
50. Onishi T, Hruska, K. Expression of p27Kip1 in osteoblast-like cells during differentiation with parathyroid hormone. ***Endocrinology*** 1997; 138: 1995–2004.
51. Spirin KS, Simpson JF, Takeuchi S, Kawamata N, Miller CW, Koeffler HP. p27/Kip1 mutation found in breast cancer. ***Cancer Res*** 1996; 56: 2400–2404.
52. Catzavelos C, Bhattacharya N, Ung YC, Wilson JA, Roncari L, Sandhu C et al. Decreased levels of the cell-cycle inhibitor p27Kip1 protein: prognostic implications in primary breast cancer. ***Nat Med*** 1997; 3: 227-230.
53. Fredersdorf S, Burns J, Milne AM, Packham G, Fallis L, Gillett CE, et al. High level expression of p27(kip1) and cyclin D1 in some human breast cancer cells: inverse correlation between the expression of p27(kip1) and degree of malignancy in some human breast and colorectal cancers. ***Proc Natl Acad Sci USA*** 1997; 94: 6380–6385.
54. Tan P, Cady B, Wanner M, Worland P, Cukor B, Magi-Galluzzi C et al. The cell cycle inhibitor p27 is an independent prognostic marker in small (T1a,b) invasive breast carcinoma. ***Cancer Res*** 1997; 57: 1259-1263.
55. Watts CK, Brady A, Sarcevic B, deFazio A, Musgrove EA, Sutherland RL. Antiestrogen inhibition of cell cycle progression in breast cancer cells is associated with inhibition of cyclin-dependent kinase activity and decreased retinoblastoma protein phosphorylation. ***Mol Endocrinol*** 1995; 9: 1804–1813.

56. Signoretti S, Di Marcotullio L, Richardson A, Ramaswamy S, Isaac B, Rue M, et al. Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer. ***J Clin Invest*** 2002; 110: 633–641.
57. Yamauchi H, Stearns V, Hayes D. When is a tumor marker ready for prime time? A case study of c-erb-B2 as a predictive factor in breast cancer. ***J Clin Oncol*** 2001; 19: 2334-2356.
58. Cooke T, Reeves J, Lannigan A, Stanton P. The value of human epidermal growth factor receptor-2 (HER2) as a prognostic factor. ***Eur J Cancer*** 2001; 37: s3-s10.
59. Normanno N, Bianco C, DeLuca A, Maeillo MR, Salomon DS. Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment. ***Endocrine Relat Cancer*** 2003; 10: 1-21.
60. Baulida J, Kraus MH, Alimandi M, Di Fiore PP, Carpenter G. All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired. ***J Biol Chem*** 1996; 271: 5251-5257.
61. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor dimerisation in development and cancer. ***EMBO J*** 2000; 19: 3159-3167.
62. Hamilton A, Piccart M. The contribution of molecular markers to the prediction of response in the treatment of breast cancer: a review of the literature on Her-2, p53 and bcl-2. ***Ann Oncol*** 2000; 11: 647-663.
63. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, et al. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. ***Breast Cancer Res*** 2003; 6: 24-30.
64. Shawver LK, Slamon D, Ullrich A. Smart drugs: tyrosine kinase inhibitors in cancer therapy. ***Cancer Cell*** 2002; 2: 117-123.
65. Alokail MS. Transient transfection of epidermal growth factor receptor gene into MCF7 breast ductal carcinoma cell line. ***Cell Biochem Funct*** 2005; 23: 157-161.
66. Neve RM, Sutterluty H, Pullen N, Lane HA, Daly JM, Krek W, et al. Effects of oncogenic ErbB2 on G1 cell cycle regulators in breast tumor cells. ***Oncogene*** 2000; 19: 1647-1656.
67. Naidu R, Yadav M, Nair S, Kutty KK. Immunohistochemical analysis of p53 expression in primary breast carcinomas. ***Anticancer Res*** 1998; 18: 65-70.
68. Chow SN, Chen M, Chen PJ, Chen RJ, Chien CH. Cell cycle analysis and detection of proliferative cell nuclear antigen of the endometrium after hormone replacement therapy. ***Maturitas*** 2001; 39: 227-237.
69. Guy CT, Muthuswamy SK, Cardiff RD, Soriano P, Muller WJ. Activation of the c-Src tyrosine kinase is required for the induction of mammary tumors in transgenic mice. ***Genes Dev*** 1994; 8: 23-32.
70. Prigent SA, Gullick WJ. Identification of c-erbB-3 binding sites for phosphatidylinositol 3'-kinase and SHC using an EGF receptor/c-erbB-3 chimera. ***EMBO J*** 1994; 13: 2831-2841.
71. Gompel A, Chaouat M, Hugol D, Forgez P. Steroidal hormones and proliferation, differentiation and apoptosis in breast cells. ***Maturitas*** 2004; 49: 16-24.
72. Gustafsson JA. Estrogen receptor beta --a new dimension in estrogen mechanism of action. ***J Endocinol*** 1999; 163: 379-383.
73. Saji S, Jensen EV, Nilsson S, Rylander M, Warner M, Gustafsson JA. Estrogen receptors and in the rodent mammary gland. ***Proc Natl Acad Sci USA*** 2000; 97: 337-342.
74. Nilsson S, Maketa S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanism of estrogen action. ***Physiol Rev*** 2001; 81: 1535-1565.
75. Dong L, Wang W, Wang F, Stoner M, Reed JC, Harigai M, et al. Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells. ***J Biol Chem*** 1999; 274: 32099-32107.
76. Zheng W, Lu J, Zheng J, Hu F, Ni C. Variation of ER status between primary and metastatic breast cancer and relationship to p53 expression. ***Steroids*** 2001; 66: 905-910.
77. Shang Y, Hu X, DiRenzo J, Lzar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. ***Cell*** 2000; 103: 843-852.
78. Gotteland M, May E, May LE, Contesso G, Delarue JC, Mouriesse H. Estrogen receptor in human breast cancer. The significance of a new prognostic factor based on both ER protein and ER mRNA. ***Cancer*** 1994; 74: 864-869.
79. Osborne CK. Steroid hormone receptors in breast cancer management. ***Breast Cancer Res Treat*** 1998; 51: 227-238.
80. Roger P, Sahla ME, Makela S, Gustafsson JA, Baldet P, Rochefort H. Decreased expression of estrogen receptor beta protein in proliferative preinvasive mammary tumors. ***Cancer Res*** 2001; 61: 2537-2541.
81. Caleffi M, Teague MW, Jensen RA, Vnencak-Jones CL, Dupont WD, Parl FF. P53 gene mutations and steroid receptor status in breast cancer, clinicopathologic correlations and prognostic assessment. ***Cancer*** 1994; 73: 2147-2156.
82. Angeloni SV, Martin MB, Garcia-Moralis P, Castro-Galachi MD, Ferragut JA, Saceda M. Regulation of estrogen receptor-ß expression by the tumor suppressor gene p53 in MCF-7 cells. ***J Endocrinol*** 2004; 180: 497-504.
83. Liu G, Schwartz JA, Brooks SC. Estrogen receptor protects p53 from deactivation by human double minute-2. ***Cancer Res*** 2000; 60: 1810-1814.
84. Maeda K, Tsuda H, Hashuguchi Y, Ymamoto K, Inoue T, Ishiko O, et al. Relationship between p53 pathway and estrogen receptor status in endometrioid-type endometrial cancer. ***Hum Pathol*** 2002; 33: 386-391.
85. Clark GM, McGuire WL. Steroid receptors and other prognostic factors in primary breast cancer. ***Semin Oncol*** 1988; 15 (2 Suppl 1): 20-25.
86. Nicholson S, Sainsbury JR, Halcrow P, Chambers P, Farndon JR, Harris AL. Expression of epidermal growth factor receptors associated with lack of response to endocrine therapy in recurrent breast cancer. ***Lancet*** 1989; 8631: 182-185.
87. Gusterson BA. Identification and interpretation of epidermal growth factor and c-erbB-2 overexpression. ***Eur J Cancer*** 1992; 28: 263-267.