Molecular Markers of Multiple Drug Resistance in Breast Cancer

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Key Words

Breast cancer · Multiple drug resistance · P-glycoprotein · Multidrug resistance-associated protein · Lung resistance protein

Abstract

Breast cancer is a significant health problem in terms of both morbidity and mortality, with approximately 12% of women directly affected by this disease. Chemotherapy, given to patients with earlier stage disease, has a good survival impact and may contribute to cure. The failure of chemotherapeutic drugs to eradicate cancer cells in more advanced disease states may be due to intrinsic or acquired drug resistance, including multiple drug resistance. The drug resistance observed in breast cancer patients is likely to be multifactorial, involving mechanisms such as altered expression and/or activity of drug efflux pumps, nuclear DNA-binding enzymes, metabolizing and conjugating enzymes, and mismatch repair deficiency. More extensive transcriptomic and proteomic analyses of breast tumour and normal biopsies, followed by functional genomic studies in relevant cell line models, should increase our understanding of

this phenomenon and lead to therapies being individualized for identifiable subgroups of breast cancer patients.

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Introduction

Breast cancer is a leading cause of cancer deaths in women all over the world [1]. Each year, this disease is diagnosed in >1,000,000 people – having increased from an estimated 572,100 cases in 1980 – and is the cause of >400,000 deaths. The median age for patients with breast cancer is 65 years [2]; however, this disease may affect women of all ages. Breast cancer is not restricted to the female population – approximately 1% of all cases is diagnosed in men.

Breast tumours are currently diagnosed and subclassified, based on a limited number of clinical and gross pathological features and immunochemical markers, although patients diagnosed with the same stage of cancer may have a completely different course of disease, suggesting that morphologically similar tumours may have different molecular properties [3]. While some progress in individualizing therapy based on molecular diagnosis has been made, e.g., treatment of oestrogen receptor-positive cancers with hormone-containing therapy [4] and treat-

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ment of Her2/*neu*-positive cancers with trastuzumab (herceptin)-containing treatment regimes [5, 6], due to our poor understanding of the complex biology of breast cancer, the majority of patients receive some form of non-individualized chemotherapy, but as few as 50% benefit from this.

Drug Resistance in Breast Cancer

Cytotoxic drugs play an important role in the treatment of breast cancer [7–9]. However, chemotherapy resistance, whether inherent or acquired, is a major problem in the management of breast cancer. Patients refractory to chemotherapy often exhibit resistance to multiple cytotoxic agents, often of differing structures and differing functions. The basis for the multiple drug resistance (MDR) is likely to be multifactorial and heterogeneous [8, 10, 11], with many molecular mechanisms potentially contributing to the drug resistance phenotype.

Drug Efflux Pumps

Several of the transmembrane drug efflux pumps belong to the ATP-binding cassette (ABC) transmembrane protein superfamily that utilizes energy from ATP hydrolysis to translocate substrates across cell membranes [8, 12]. This mechanism of drug resistance may be clinically relevant in breast cancer patients. ABC family members (<http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html> and <http://www.med.rug.nl/mdl/humanabc.htm>) [13–15] involved in MDR include multiple drug resistance 1/P-glycoprotein (MDR1/Pgp) (ABCB1), multidrug resistance-associated protein (MRP) family members MRP1 (ABCC1), MRP2 (ABCC2), MRP3 (ABCC3), MRP4 (ABCC4), MRP5 (ABCC5), MRP6 (ABCC6), and breast cancer resistance protein (BCRP) (ABCG2).

MDR1/Pgp Expression

The MDR1 gene encodes Pgp, a 170-kDa protein consisting of 12 transmembrane domains and two ATP-binding domains [16, 17]. Drugs to which MDR1/Pgp confers resistance include anthracyclines [18], epipodophyllotoxins, vinca alkaloids, geldanamycin [15] and taxanes [19]; apparently, methotrexate, 5-fluorouracil, camptothecins and hydroxyurea are not substrates for MDR1/Pgp [15].

The relevance of MDR1/Pgp gene expression in breast cancer has been extensively investigated, with contradictory results ranging from 0 to 100% expression in tumour

cells [8, 18] and with conflicting reports as to its prognostic/predictive relevance. A meta-analysis of such studies reported MDR1/Pgp protein to be expressed in approximately 41% of breast tumours and associated with poor response to treatment [20]. Similarly, in a study specifically of chemotherapy-naïve breast tumours, MDR1/Pgp protein expression (found in 37% of cases) was reported to be significantly associated with shortened disease-free survival (DFS), when compared with those that were MDR1/Pgp negative [21]. In contrast, overexpression of MDR1/Pgp protein in locally advanced breast cancer following neoadjuvant chemotherapy – cyclophosphamide + doxorubicin + 5-fluorouracil, taxotere + doxorubicin, or cyclophosphamide + methotrexate + 5-fluorouracil (CMF) – showed no correlation with response to chemotherapy [22]. Similarly, our study of 177 invasive breast carcinomas showed no association between MDR1/Pgp protein expression at diagnosis and patients' outcome [23]. Of course, such studies can assess only protein levels, not activity.

MRP1 Expression

The MRP subfamily is comprised of nine members that transport structurally diverse lipophilic anions and hydrophilic peptides [24]. MRP1 gene encodes a 190-kDa transmembrane protein associated with both cell membrane and intracellular membrane expression [12]. MRP1 confers resistance to anthracyclines, epipodophyllotoxins, methotrexate, and the vinca alkaloid vincristine; however, it apparently confers no/only low-level resistance to vinblastine [25]. In contrast to MDR1/Pgp, taxanes are poor substrates for MRP1 and its overexpression in cell lines has been shown not to confer resistance to cisplatin and mitoxantrone [26].

In general, there appears to be a correlation between MRP1 protein expression and poor outcome for breast cancer patients, although a direct causal role for MRP1 in clinical drug resistance remains to be determined [12]. In a study of 100 primary breast cancer tumours, the absence of MRP1 protein was found to be significantly associated with increased DFS and overall survival (OS), compared with those with MRP1 tumours [27]. In our study of 177 invasive breast carcinomas, we found no association between MRP1 expression at diagnosis (presence/absence and <25% MRP1 expression versus ≥25% MRP1 expression) and either DFS or OS. However, a significant correlation was observed between MRP1 protein expression in ≥25% tumour cells and shorter DFS or OS for patients with high-grade tumours (grade III) who received adjuvant CMF. Multivariate analysis iden-

tified MRP1 expression in this subgroup to be an independent poor prognostic factor for both DFS and OS [23]. Furthermore, the relevance of MRP1 in patients treated with CMF has more recently been confirmed in a study of 516 premenopausal, hormone receptor-positive breast cancer patients with stage I and II disease, where MRP1 expression was found to be an independent predictor of shorter relapse-free survival and OS [28].

Studies investigating the relevance in breast cancer MDR of more recently described members of the MRP family, such as MRP2 [29] and MRP8 [30], have commenced. An analysis of MRP1, MRP2 and MRP3 mRNA expression in untreated and post-neoadjuvant anthracycline-based tumours indicated expression of all three family members in 100% specimens studied, with no significant increase in levels after chemotherapy. Corresponding proteins, however, were not detectable [31]. MRP7 may confer resistance to taxanes [32]. More extensive studies are now necessary to investigate the clinical relevance (in any) to breast cancer of expression of all MRP family members.

Role of BCRP

BCRP (also known as mitoxanthrone resistance gene) is a 72-kDa half-transporter consisting of six transmembrane domains with one ATP-binding domain [33]. BCRP transports a number of anticancer drugs including mitoxanthrone, methotrexate, topotecan, irinotecan, camptothecin-derived and indolocarbazole topoisomerase I inhibitors, flavopiridol, and quinazoline ErbB1 inhibitors, and its transportation of anthracyclines depends on a mutation at codon 482 [12, 34, 35]. However, although described as 'breast cancer resistance protein', the definitive role of BCRP in clinical drug resistance in breast cancer is still unclear [36].

Lung Resistance Protein

Lung resistance protein (LRP; also known as major vault protein), a 110-kDa protein, is not an ABC transporter, but its expression is frequently detected at high levels in drug-resistant cell lines and tumour specimens. Expression of LRP has been associated with resistance to platinum, alkylating agents [37] and doxorubicin [38]. An immunohistochemistry study of LRP in 99 primary breast carcinomas indicated low-level expression in 20%, intermediate-level expression in 47%, and high-level expression in 21% of cases (LRP was undetected in 12% of these breast carcinomas). However, expression was not significantly associated with clinicopathological characteristics evaluated, including patients' DFS or OS [39].

Similarly, a study of 80 locally advanced breast carcinomas showed no correlation between LRP protein expression and clinical parameters evaluated [40].

Other Mechanisms of MDR in Breast Cancer

In addition to drug efflux pumps that may contribute to MDR by reduction in the intracellular accumulation of anticancer drugs (as a result of both increasing drug efflux and/or decreasing drug uptake and drug sequestration in subcellular organelles), other mechanisms of drug resistance have been described in breast cancer. These include alterations in drug targets – e.g., topoisomerase enzymes [41–43] and activation of detoxifying systems including glutathione/glutathione S-transferases [44–46] and cytochrome P450 enzymes [47, 48] – increased repair of drug-induced DNA damage, disruption in cell signalling, alterations in factors involved in cell cycle control, and inhibition of apoptosis [12]. Due to space limitations, an extensive review of these MDR mechanisms is beyond the scope of this manuscript.

Future Prospects

Recent studies involving the application of expression microarrays, including the work of van't Veer et al. [49] identifying 70 discriminatory genes between metastatic and non-metastatic breast carcinomas and studies reported by Wang et al. [50] identifying a 76 gene signature relevant to distant metastasis of lymph node-negative primary breast tumours, indicate the importance and relevance of such molecular profiling analyses. More extensive microarray and proteomics profiling of larger and different cohorts of breast clinical specimens should facilitate the future development of rational combination chemotherapy regimes, in which cytotoxic drugs may be administered with newer targeted therapies, as relevant, to individuals or particular subgroups of breast cancer patients.

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References

- Chintamani, Singhal V, Singh JP, Lyall A, Saxena S, Bansal A: Is drug-induced toxicity a good predictor of response to neo-adjuvant chemotherapy in patients with breast cancer? - A prospective clinical study. *BMC Cancer* 2004;4:48.
- Crown J, Dieras V, Kaufmann M, von Minckwitz G, Kaye S, Leonard R, Marty M, Misset JL, Osterwalder B, Piccart M: Chemotherapy for metastatic breast cancer - Report of a European expert panel. *Lancet Oncol* 2002;3:719-726.
- Schmidt U, Begley CG: Cancer diagnosis and microarrays. *Int J Biochem Cell Biol* 2003;35:119-124.
- Choueiri TK, Alemany CA, Abou-Jawde RM, Budd GT: Role of aromatase inhibitors in the treatment of breast cancer. *Clin Ther* 2004;26:1199-1214.
- Bell R, Verma S, Untch M, Cameron D, Smith I: Maximizing clinical benefit with trastuzumab. *Semin Oncol* 2004;31(suppl 10):35-44.
- Esteve FJ: Monoclonal antibodies, small molecules, and vaccines in the treatment of breast cancer. *Oncologist* 2004;9(suppl 3):4-9.
- Sangrajrang S, Fellous A: Taxol resistance. *Chemotherapy* 2000;46:327-334.
- Leonessa F, Clarke R: ATP binding cassette transporters and drug resistance in breast cancer. *Endocr Relat Cancer* 2003;10:43-73.
- Ikeda T, Jinno H, Kitajima M: The evolution of primary chemotherapy in breast cancer treatment. *Breast Cancer* 2004;11:148-155.
- Giai M, Biglia N, Sismondi P: Chemoresistance in breast tumors. *Eur J Gynaecol Oncol* 1991;12:359-373.
- Clynes M, Daly C, NicAmhlaoibh R, Cronin D, Elliott C, O'Connor R, O'Doherty T, Connolly L, Howlett A, Scanlon K: Recent developments in drug resistance and apoptosis research. *Crit Rev Oncol Hematol* 1998;28:181-205.
- Filipits M: Mechanisms of cancer: multidrug resistance. *Drug Discov Today* 2004;1:229-234.
- Wijnholds J: Drug resistance caused by multidrug resistance-associated proteins. *Novartis Found Symp* 2002;243:69-79.
- Leonard GD, Fojo T, Bates SE: The role of ABC transporters in clinical practice. *Oncologist* 2003;8:411-424.
- Szakacs G, Annereau JP, Lababidi S, Shankavaram U, Arciello A, Bussey KJ, Reinhold W, Guo Y, Kruh GD, Reimers M, Weinstein JN, Gottesman MM: Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells. *Cancer Cell* 2004;6:129-137.
- Juliano RL, Ling V: A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976;455:152-162.
- Gottesman MM, Pastan I: Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993;62:385-427.
- Kroger N, Achterath W, Hegewisch-Becker S, Moss K, Zander AR: Current options in treatment of anthracycline-resistant breast cancer. *Cancer Treat Rev* 1999;25:279-291.
- Duan Z, Lamendola DE, Duan Y, Yusuf RZ, Seiden MV: Description of paclitaxel resistance-associated genes in ovarian and breast cancer cell lines. *Cancer Chemother Pharmacol* 2005;55:277-285.
- Trock BJ, Leonessa F, Clarke R: Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. *J Natl Cancer Inst* 1997;89:917-931.
- Tsukamoto F, Shiba E, Taguchi T, Sugimoto T, Watanabe T, Kim SJ, Tanji Y, Kimoto Y, Izukura M, Takai SI: Immunohistochemical detection of P-glycoprotein in breast cancer and its significance as a prognostic factor. *Breast Cancer* 1997;25:259-263.
- Schneider J, Lucas R, Sanchez J, Ruibal A, Tejerina A, Martin M: Modulation of molecular marker expression by induction chemotherapy in locally advanced breast cancer: correlation with the response to therapy and the expression of MDR1 and LRP. *Anticancer Res* 2000;20:4373-4377.
- Larkin A, O'Driscoll L, Kennedy SM, Purcell R, Moran E, Crown J, Parkinson M, Clynes M: Investigation of MRP-1 protein and MDR-1 Pgp expression in invasive breast cancer: a prognostic study. *Int J Cancer* 2004;112:286-294.
- Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM, Deeley RG: Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 1992;258:1650-1654.
- Cole SP, Sparks KE, Fraser K, Loe DW, Grant CE, Wilson GM, Deeley RG: Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. *Cancer Res* 1994;54:5902-5910.
- Almquist KC, Loe DW, Hipfner DR, Mackie JE, Cole SP, Deeley RG: Characterization of the M(r) 190,000 multidrug resistance protein (MRP) in drug-selected and transfected human tumor cell. *Cancer Res* 1995;55:102-110.
- Filipits M, Malayeri R, Suchomel RW, Pohl G, Stranzl T, Dekan G, Kaider A, Stiglbauer W, Depisch D, Pirker R: Expression of the multidrug resistance (MRP1) in breast cancer. *Anticancer Res* 1999;19:5043-5049.
- Filipits M, Pohl G, Rudas M, Dietze O, Lax S, Grill R, Pirker R, Zielinski CC, Hausmaninger H, Kubista E, Samonig H, Jakesz R: Clinical role of multidrug resistance protein 1 expression in chemotherapy resistance in early-stage breast cancer: the Australian Breast and Colorectal Cancer Study Group. *J Clin Oncol* 2005;23:1161-1168.
- Kool M, De Haas M, Scheffer GL, Scheper RJ, Van Eijk MJT, Juijn JA, Baas F, Borst P: Analysis of expression of CMOAT (MRP2), MRP3, MRP4 and MRP5 homologues of the multidrug resistance-associated protein gene (MRP1) in human cancer cell lines. *Cancer Res* 1997;57:3537-3547.
- Bera TK, Lee S, Salvatore G, Lee B, Pastan I: MRP8, a new member of ABC transporter superfamily, identified by EST database mining and gene prediction program, is highly expressed in breast cancer. *Mol Med* 2001;7:509-516.
- Faneyte IF, Kristel PM, van de Vijver MJ: Multidrug resistance associated genes MRP1, MRP2 and MRP3 in primary and anthracycline exposed breast cancer. *Anticancer Res* 2004;24:2931-2939.
- Hopper-Borge E, Chen ZS, Shehavelva I, Belinsky MG, Kruh GD: Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): resistance to docetaxel. *Cancer Res* 2004;64:4927-4930.
- Doyle LA, Yang W, Abruzzo LV, Krognmann T, Gao Y, Rishi AK, Ross DD: A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* 1998;95:15665-15670.
- Robey RW, Medina-Perez WY, Nishiyama K, Lahusen T, Miyake K, Litman T, Senderowicz AM, Ross DD, Bates SE: Overexpression of the ATP-binding cassette half-transporter, ABCG2 (MXR/BCRP/ABCP1), in flavopiridol-resistant human breast cancer cells. *Clin Cancer Res* 2001;7:145-152.
- Doyle LA, Ross DD: Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). *Oncogene* 2003;22:7340-7358.
- Lage H, Dietel M: Effect of the breast-cancer resistance protein on atypical multidrug resistance. *Lancet Oncol* 2000;1:169-175.
- Izquierdo MA, van der Zee AG, Vermorken JB, van der Valk P, Belien JA, Giaccone G, Scheffer GL, Flens MJ, Pinedo HM, Kenemans P, et al: Drug resistance-associated marker Lrp for prediction of response to chemotherapy and prognosis in advanced ovarian carcinoma. *J Natl Cancer Inst* 1995;87:1230-1237.
- Cheng SH, Lam W, Lee AS, Fung KP, Wu RS, Fong WF: Low-level doxorubicin resistance in benzo[a]pyrene-treated KB-3-1 cells is associated with increased LRP expression and altered subcellular drug distribution. *Toxicol Appl Pharmacol* 2000;164:134-142.
- Pohl G, Filipits M, Suchomel RW, Stranzl T, Depisch D, Pirker R: Expression of the lung resistance protein (LRP) in primary breast cancer. *Anticancer Res* 1999;19:5051-5055.
- Rudas M, Filipits M, Taucher S, Stranzl T, Steger GG, Jakesz R, Pirker R, Pohl G: Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. *Breast Cancer Res Treat* 2003;81:149-157.

- 41 Park K, Kim J, Lim S, Han S: Topoisomerase II-alpha (topoII) and HER2 amplification in breast cancers and response to preoperative doxorubicin chemotherapy. *Eur J Cancer* 2003;39:631-634.
- 42 Koren R, Rath-Wolfson I, Ram E, Itzhac OB, Schachter B, Klein B, Gal R, Dreznik Z: Prognostic value of Topoisomerase II in female breast cancer. *Oncol Rep* 2004;12:915-919.
- 43 Durbeeq V, Paesmans M, Cardoso F, Desmedt C, Di Leo A, Chan S, Friedrichs K, Pinter T, Van Belle S, Murray E, Bodrogi I, Walpole E, Lesperance B, Korec S, Crown J, Simmonds P, Perren TJ, Leroy JY, Rouas G, Sotiriou C, Piccart M, Larsimont D: Topoisomerase-II alpha expression as a predictive marker in a population of advanced breast cancer patients randomly treated either with single-agent doxorubicin or single-agent docetaxel. *Mol Cancer Ther* 2004;3:1207-1214.
- 44 Kelley MK, Engqvist-Goldstein A, Montali JA, Wheatley JB, Schmidt DE Jr, Kauvar LM: Variability of glutathione S-transferase isoenzyme patterns in matched normal and cancer human breast tissue. *Biochem J* 1994;304:843-848.
- 45 Gilbert L, Elwood LJ, Merino M, Masood S, Barnes R, Steinberg SM, Lazarous DF, Pierce L, d'Angelo T, Moscow JA, et al: A pilot study of pi-class glutathione S-transferase expression in breast cancer: correlation with estrogen receptor expression and prognosis in node-negative breast cancer. *J Clin Oncol* 1993;11:49-58.
- 46 Moskatelo D, Benjak A, Laketa V, Polanc S, Kosmrlj J, Osmak M: Cytotoxic effects of diazenes on tumor cells in vitro. *Chemotherapy* 2002;48:36-41.
- 47 Galant C, Gala JL, Van Den Berge V, Berliere M, Haumont E, Horsmans Y: Immunolocalisation of cytochrome P-450 3A enzymes in human breast carcinoma: relationship with tumour differentiation and steroid receptors. *Pharmacol Toxicol* 2001;88:142-146.
- 48 Miyoshi Y, Ando A, Takamura Y, Taguchi T, Tamaki Y, Noguchi S: Prediction of response to docetaxel by CYP3A4 mRNA expression in breast cancer tissues. *Int J Cancer* 2002;97:129-132.
- 49 van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marion MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH: Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530-536.
- 50 Wang Y, Klijn JGM, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EMJJ, Atkins D, Foekens JA: Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005;365:671-679.