General mechanisms of drug resistance in metastatic breast cancer: focus on chemotherapy

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Peek before you treat?

• “empiricism” – fundamental strategy of recommending the same therapy to a group of patients with similar cancer conditions (eg, stage IV lung cancer)

➢ Toxic effects are more or less guaranteed, but clinical benefits are not

• Most cancers are highly complex; even the same tumor type can harbor hundreds of different genetic abnormalities

• The number of somatic mutations in one breast cancer can be more than 1,000, and up to 100 of these can be unique compared with those of another breast cancer
What possible solutions are there?
- concept of Chemotherapy Sensitivity and Resistance Assays (CSRAs)

• “We need new tools to measure sensitivity in a greater percentage of patients and need different approaches.” ¹

• ASCO Recommendations-2011: “The use of CSRAs to select chemotherapeutic agents for individual patients is not recommended outside of the clinical trial setting. Oncologists should make chemotherapy treatment recommendations on the basis of published reports of clinical trials and a patient’s health status and treatment preferences.” ²

1. Daniel D. Von Hoff: Karnofsky Memorial Award Lecture- ASCO Annual Meeting 2010
The Science of Chemotherapy - cause of drug resistance

Ian Tannock’s PhD Project
Using tritiated thymidine autoradiography, showed solid tumors proliferate more slowly further away from blood vessels.

THE RELATION BETWEEN CELL PROLIFERATION AND THE VASCULAR SYSTEM IN A TRANSPLANTED MOUSE MAMMARY TUMOUR

I. F. TANNOCK
From the Biophysics Department, Institute of Cancer Research, Clifton Avenue, Belmont, Sutton, Surrey

Received for publication January 15, 1968
Multidrug resistance (MDR)

- MDR is a phenomenon whereby tumor cells in vitro that have been exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds.
- MDR occurs intrinsically in some cancers without previous exposure to chemotherapy agents.¹

To date, despite intense effort, there are no agents clinically available that are capable of overcoming multidrug resistance.

# Mechanisms of resistance

**Major known mechanisms of drug resistance in metastatic breast cancer**

<table>
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<th>Mechanisms of Drug Resistance</th>
<th>Examples</th>
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<td>Drug efflux mechanisms</td>
<td>ABC transporters: P-gp, MRP1, BCRP</td>
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<tr>
<td>Microtubule dysfunction</td>
<td>Increased expression of β-III tubulin, failure to induce apoptotic signal</td>
</tr>
<tr>
<td>HER2</td>
<td>Alternative signaling through EGFR members, activation of PI3K, loss of PTEN</td>
</tr>
<tr>
<td>PI3K pathway</td>
<td>Activating mutations, amplification of Akt, loss of PTEN, Kras activation</td>
</tr>
<tr>
<td>DNA repair pathways</td>
<td>Loss of heterozygosity, somatic mutations restoring BRCA1 function</td>
</tr>
<tr>
<td>Breast Cancer Stem Cells</td>
<td>MDR, anti-apoptotic molecules</td>
</tr>
<tr>
<td>Apoptotic pathway</td>
<td>PTEN loss, Bcl-2 expression</td>
</tr>
</tbody>
</table>

P-gp, P-glycoprotein; MRP1, multidrug-resistant protein; BCRP, breast cancer resistant protein; EGFR, epidermal growth receptor; PI3K, phosphoinositol 3-kinase; PTEN, phosphate and tensin homologue
Drug efflux mechanism

- ATP-binding cassette (ABC) family of proteins function to translocate a variety of compounds across cell membranes using energy from adenosine triphosphate hydrolysis; at least 48 ABC transporter genes divided in seven different families (A-G)
- Overexpression of the transport proteins: P-gp, MRP-multidrug resistance proteins, BCRP-breast cancer resistance protein

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein/alias</th>
<th>Chemotherapeutic drugs effluxed by transporter</th>
<th>Other drugs and substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA2</td>
<td>ABCA2</td>
<td>Estramustine</td>
<td>–</td>
</tr>
<tr>
<td>ABCB1</td>
<td>PGP/MDR1</td>
<td>Colchicine, doxorubicin, etoposide, vinblastine, paclitaxel</td>
<td>Digoxin, saquinivir</td>
</tr>
<tr>
<td>ABCC1</td>
<td>MRP1</td>
<td>Doxorubicin, daunorubicin, vincristine, etoposide, colchicine, camptothecins, methotrexate</td>
<td>Rhodamine</td>
</tr>
<tr>
<td>ABCC2</td>
<td>MRP2</td>
<td>Vinblastine, cisplatin, doxorubicin, methotrexate</td>
<td>Sulfinpyrazone</td>
</tr>
<tr>
<td>ABCC3</td>
<td>MRP3</td>
<td>Methotrexate, etoposide</td>
<td>–</td>
</tr>
<tr>
<td>ABCC4</td>
<td>MRP4</td>
<td>6-mercaptopurine, 6-thioguanine and metabolites, methotrexate</td>
<td>PMEA, cAMP, cGMP</td>
</tr>
<tr>
<td>ABCC5</td>
<td>MRP5</td>
<td>6-mercaptopurine, 6-thioguanine and metabolites</td>
<td>PMEA, cAMP, cGMP</td>
</tr>
<tr>
<td>ABCC6</td>
<td>MRP6</td>
<td>Etoposide</td>
<td>–</td>
</tr>
<tr>
<td>ABCC11</td>
<td>MRP8</td>
<td>5-fluorouracil</td>
<td>PMEA, cAMP, cGMP</td>
</tr>
<tr>
<td>ABCG2</td>
<td>MXR/BCRP</td>
<td>Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, imatinib, methotrexate</td>
<td>Pheophorbide A, Hoechst 33342, rhodamine</td>
</tr>
</tbody>
</table>

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanine monophosphate; MDR, multidrug resistance; MRP, multidrug-resistance-associated protein; MXR, mitoxantrone resistance protein; PMEA, 9-[2-(phosphonomethoxy)ethyl]adenine.

Source: Nat Rev Cancer © 2005 Nature Publishing Group
P-gp in breast cancer

• “P” – Permeability glycoprotein; membrane protein encoded by ABCB1 (MDR1) gene

• Anticancer drugs, substrates of P-gp: antracyclines, vinca alkaloids, taxanes; MTX and 5FU are poor P-gp substrates

• Meta analysis: ~40% BC express P-gp irrespective of the stage ¹

• Prior exposure to chemotherapy or hormonal therapy increase expression ~1.8-fold ²

• Neo-adjuvant CT with anthracyclines ot taxanes: no substantial upregulation of P-gp ⁴

• Only drastic transcriptional upregulation of P-gp result in transporter levels sufficient to resistance

Pumps - conclusion

• Role of ABC transporters in drug resistance in real tumors is limited value
• Effective inhibitors of P-gp have shown only limited effects in clinical trials $^{1,2}$
  ➢ Third generation P-gp inhibitor: 5-fold increase in brain uptake of paclitaxel by combinatorial treatment with elacridar$^{3}$
• Evaluation of the MDR expression may serve in the future as an additional stratification for identifying high risk cancers

Resistance to microtubule inhibitors

- Microtubule- critical components of the cytoskeleton and cell division; composed of α and β tubulin heterodimers along with microtubule associated proteins
- Disruption of microtubule processes through stabilization (taxane family) or destabilization (vinca family)
- **Resistance**: presence of the MDR phenotype or alteration in the microtubule molecular target
Microtubule molecular target alterations

- altered expression of tubulin isotypes; overexpression of β3 (weak) and β5 (robust)

- expression of β3 tubulin predictor of response to paclitaxel (PD in 2% vs. 38%)

- changes in microtubule-associated proteins - MAPs: stathmin, MAP4, γ-actin, tau, mitotic centromere associated kinesin (MCAK)

1. Edith Perez. Mol Cancer Ther, 2009
5. Ganguly A, Cabral F. Biochim Biophys Acta, 2011
Novel Microtubule Inhibitors

- Activity in taxane resistant cancers: **epothilones** and **halichondrins**
- Ixabepilone, semisynthetic analog of epothilone B; binding to a β-tubulin subunit; unique mechanism of action
- Ixabepilone; low susceptibility to tumor resistance mechanisms (P-gp, MRP1, β-3-tubulin)

Ixabepilone Plus Capecitabine for Metastatic Breast Cancer Progressing After Anthracycline and Taxane Treatment

- Ixabepilone derived from myxobacteria sorangium Cellulosum along the Zambezi River

Eva S. Thomas et al. JCO 2007;25:5210-5217
Halichondrins
Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer

Eribulin:
bind to β-tubulin at a site close to the vinca site, inhibiting polymerization and separating microtubules into nonfunctional aggregates

EMBRACE Study Results

Phase III Open-Label Randomized Study of Eribulin Mesylate Versus Capecitabine in Patients With Locally Advanced or Metastatic Breast Cancer Previously Treated With an Anthracycline and a Taxane

Kaplan-Meier curve for (A) overall survival and (B) progression-free survival (independent review; intent-to-treat population).

Peter A. Kaufman et al. JCO 2015;33:594-601
Breast cancer stem cells

Excise cancer from Breast

Isolate Cancer cells

Inject mice

10^3 cells 10^4 cells 10^5 cells 10^6 cells

No Tumor No Tumor No Tumor Tumor

Label cells with antibodies for the cell surface molecules CD24 and CD44 – sort by flow cytometry.

200 of these form a tumor in a NOD/SCID mouse
20,000 of these fail to form a tumor in a NOD/SCID mouse

# Models to explain the origin and evolution of cancer

<table>
<thead>
<tr>
<th>Model</th>
<th>Organization</th>
<th>Description</th>
<th>Tumorigenic cell frequency</th>
<th>Tumorigenic cell phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal (stochastic) evolution model</td>
<td>Not hierarchical, natural competition between clones</td>
<td>The acquisition of dominant traits results from randomly occurring genetic and epigenetic events</td>
<td>High</td>
<td>Variable</td>
</tr>
<tr>
<td>Cancer stem cell model</td>
<td>Hierarchical, rigid separation between tumorigenic and nontumorigenic cells</td>
<td>Only cancer stem cells are tumorigenic</td>
<td>Rare (or moderate)</td>
<td>Fixed</td>
</tr>
<tr>
<td>Combined (clonal evolution–stem cell model)</td>
<td>'Dynamic stemness', the stem cell state can be acquired</td>
<td>Stem-like traits can be installed in noncancer stem cells by peculiar conditions existing within the microenvironment</td>
<td>Fluctuating</td>
<td>Fixed and variable</td>
</tr>
</tbody>
</table>

Maugeri-Saccà et al. Future Oncol. 2014
Cancer Stem Cells (CSC): Are They Responsible for Treatment Failure?

- Clonal evolution in the CSCs pool can be deduced from the following:
  - evidence of an increased CSC frequency in parallel with disease progression
  - therapy-induced enrichment
  - coexistence within the same tumor of different cells with tumor-forming abilities
  - genetic heterogeneity of cancer-propagating cells

1. Pece et al. Cell 2010
Different therapeutic modalities that are able to sensitize cancer stem cells to chemotherapy and radiotherapy in preclinical studies

<table>
<thead>
<tr>
<th>Strategy to increase chemo- and radio-sensitivity of cancer stem cells</th>
<th>Pathway(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeting DNA damage repair pathways</td>
<td>ATR/Chk1</td>
</tr>
<tr>
<td>Targeting apoptotic and survival pathways</td>
<td>Bcl-2-regulated apoptotic pathway, IL-4, PI3K/AKT/mTOR, EGFR/AKT/MAPK</td>
</tr>
<tr>
<td>Targeting self-renewal pathways</td>
<td>Hedgehog, Notch, TGF-β</td>
</tr>
<tr>
<td>Inducing differentiation</td>
<td>Retinoic acid, bone morphogenetic proteins</td>
</tr>
</tbody>
</table>

Maugeri-Saccà et al. Future Oncol. 2014
Targeting self-renewal pathways

- In triple-negative breast cancer, TGF-β type I receptor kinase inhibitor LY2157299 reduced the chemotherapy-resistant CSC population in vivo.

- Notch inhibition through siRNA or γ-secretase inhibitors impaired mammosphere formation and determined HER2 down regulation in tumor-initiating HER2-overexpressing cells.

- Pharmacological inhibition of the Notch signaling pathway can reduce human BCSCs in breast tumor graft models and enhance the efficacy of docetaxel.

- TAZ, a key component of the Hippo cascade, was associated with tumor initiation ability in breast cancer.

Residual disease

- Tumours may contain a fraction of **quiescent cells** that is actively kept in a (reversible) drug-tolerant state.
- Residual disease is a state of a small fraction of the tumour cells that allow these cells to avoid being killed.
- These cells are not cells that just happen to be in G1, but cells that have entered a specific quiescence programme (widespread alterations in gene expression that are reversible, allowing these cells to re-enter the cell cycle).

Piet Borst. Open Biol 2012
Pan-resistance: general consideration

- Resistance to any drug, and often also to ionizing radiation
- **Cancer cell can lost all targetable defects (carcinogenesis can be a hit-and-run process)**:\n  - DNA repair defects are mutagenic and contribute to tumor genesis but full-blown tumors do not need the defect to continue growing
  - deficiencies in homology-dependent DNA repair caused by down regulation or mutation of BRCA1/2 can be reversed in the mature tumor during treatment
  - methylation of the promoter may be reversed; second mutation

1. Borst P. Open Biol 2012
Instead of conclusion

- Why are there no obvious activated growth promoting pathways or failed cell cycle checkpoints in pan-resistant cells that can be exploited by available drugs?
- Quiescence is not an explanation: the tumor continues to proliferate, whatever the medical oncologist throws at it.