ABC Transporter Expression: Clues into Chemoresistance of Triple Negative Breast Cancers

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Background: Triple-negative breast cancer (TNBC) is an aggressive histological subtype with high rates of recurrence and metastatic disease. Intrinsic or acquired multidrug resistance facilitated by the over-expression of drug efflux pumps (ABC transporters: BCRP [ABCG2], MRP1 [ABCC1] and PGP [ABCB1]), may contribute to the aggressive nature of this disease. We examined the expression patterns of drug efflux pumps for insight on their potential role in chemoresistance of TNBC.

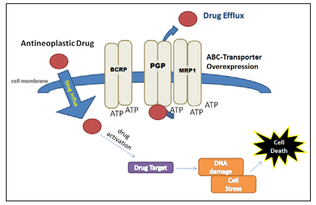
Methods: 1393 TNBC patients molecularly profiled with a commercial assay (Caris Life Sciences) were evaluated retrospectively for expression of BCRP, MRP1 and PGP by immunohistochemistry. Antibodies used: PGP (C494), BCRP (6D171) and MRP1 (33A6). IHC threshold: positive = ≥1+ and ≥10%). This data set also included metachronous paired samples from 71 TNBC patients. JMP was used to ascertain distributional differences.

Results: PGP and MRP1 positive expression rates were 8.4% (117/1393) compared to 78.2% (248/317), respectively, displaying an inverse association (p=0.0001). BCRP was over-expressed in 52% (90/173). Co-expression data for all three transporters was available for 153 patients. BCRP/MRP1 co-expression was most abundant, 39% (59/153), followed by MRP1/PGP at 4% (6/153) and BCRP/PGP at 2% (3/153). Furthermore, 12% (18/153) of TNBC exhibited positive expression for all three drug pumps (16/18 or 89% were from patients with metastatic disease) and 14% (22/153) exhibited negative expression for all three drug pumps (15/22 or 68% were from patients with metastatic disease). Interestingly, positive MRP1, BCRP and PGP, correlated with metastatic disease (p=0.05, 0.006, 0.0001). In the paired data analysis, PGP expression was absent in 77% (55/71) and retained lack of expression in subsequently profiled specimens, 3% (2/71) retained positive PGP status, 13% (9/71) lost expression of PGP and 7% (5/71) gained expression of PGP. 36/40 patients that lacked PGP expression and 8/9 patients that "lost" PGP expression, exhibited and retained expression of MRP1 in the initial and subsequently-profiled specimens.

Conclusions: Biomarker expression patterns of drug efflux pumps may provide insight to the chemoresistance phenotype observed in TNBC. Expression of MRP1 is favored in TNBC and is correlated with metastatic disease status. Although PGP expression may be absent or lost during TNBC progression, MRP1 (and BCRP) expression are almost always retained. Utilization of chemotherapies that are substrates of PGP, but not MRP1, (based on expression status) may be worthy of future investigation.

Background

- Drug resistance is a major impediment to effective treatment with antineoplastic agents
- Tumors can be intrinsically resistant prior to chemotherapy or resistance can be acquired during treatment
- Multidrug resistance (MDR) develops when tumors become resistant to many agents
- ABC* transporters show broad and overlapped specificity for substrates



ABC Transporter	List of Chemotherapies that are substrates for drug pumps
PGP (ABCB1)	doxorubicin, daunorubicin, epirubicin, colchicine, antinomycin D, etoposide, teniposide, methotrexate, mitomycin-C, paclitaxel, mitoxantrone, docetaxel, vinblastine, vincristine
MRP1 (ABCC1)	doxorubicin, daunorubicin, colchicine, topotecan, irinotecan, SN-38, methotrexate, etoposide, teniposide, vincristine, vinblastine, imatinib, gefitinib
BCRP (ABCG2)	doxorubicin, daunorubicin, epirubicin, methotrexate, topotecan, irinotecan, SN-38, etoposide, teniposide, imatinib, gefitinib

Adapted from Choi, et al. (2014). "ABC Transporters in Multidrug Resistance and Pharmacokinetics, and Strategies for Drug Development." Current Pharmaceutical Design. 20:793-807.

***ABC Transporters = ATP-Binding Cassette Transporters**

Methods

Triple negative (ER-/PR-/HER2-) breast cancer (TNBC) patients as determined by immunohistochemistry (IHC) and *in situ* hybridization (ISH) platforms, molecularly profiled with a commercial, CLIA platform (Caris Life Sciences, AZ) from 2009-2014 were assessed retrospectively, for PGP, MRP1 and BCRP expression data by IHC. 1393 patients were identified as having IHC expression profiles for at least one of the drug efflux pumps. Antibodies used included: PGP (C494), BCRP (6D171) and MRP1 (33A6). IHC thresholds (positive = ≥1+ and ≥10%) were used, based on predictive literature evidence. Slides were scored manually by board-certified pathologists, and results were reported as intensity of cell staining (0, 1+, 2+, and 3+) and percentage of tumor cells that stained positive. Expression data are represented as percent positive frequencies .

Results

Table 1. Patient and Tumor Characteristics	
Median age, range	56 [22-95]
Specimens profiled	**(n=1393)**
Primary	56% (774)
Metastatic	44% (619)
Most common sites of metastasis	**(n=619)**
Lymph nodes	31% (193)
Liver	15% (94)
Lung & Bronchus, NOS	10% (63)
Skin, NOS	7% (43)
Pleura NOS	5% (33)
Axilla, NOS	4% (25)
Brain, NOS	3.4% (21)

ABC Transporter Expression TNBCs vs. All Solid Tumors

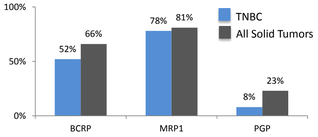


Figure 1. Percent positive expression of drug pumps in TNBC patients, compared to all solid tumors. For TNBC: n=173 for BCRP, n=317 for MRP1, n=1,393 for PGP. For all solid tumors (internal data): n=13,409 for BCRP, n=24,682 for MRP1, n=51,313 for PGP.

Results, continued

ABC Transporter Positivity Rates by IHC in TNBC

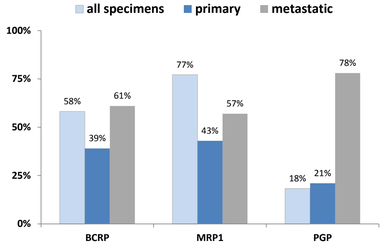


Figure 2. ABC Transporter Positivity Rates in TNBC, according to specimen site profiled (primary vs. metastatic). TNBC patients (n=153) with expression data for all 3 drug pumps (BCRP, MRP1 and PGP). The rate of positivity of PGP, MRP1 and BCRP in metastatic specimens increases for each drug pump.

Co-expression Patterns of ABC Transporters in TNBC

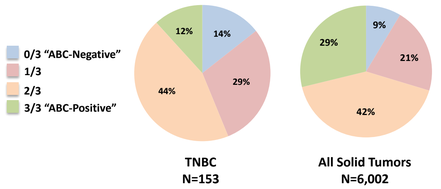


Figure 3. Patterns of Co-expression for BCRP, MRP1 and PGP in TNBC patients (left pie), compared to all solid tumors (right pie). Only 14% TNBC patients exhibited lack of drug pump expression, compared to 12% of the patients tested exhibiting co-expression of all 3 drug pumps. The majority of patients (73%) exhibit some degree of drug pump expression.

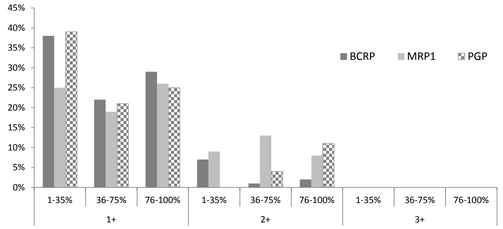


Figure 4. Staining Patterns of ABC Transporters. The majority of specimen staining occurs at 1+ staining intensity with a range of % tumor cell staining, for BCRP, MRP1 and PGP.

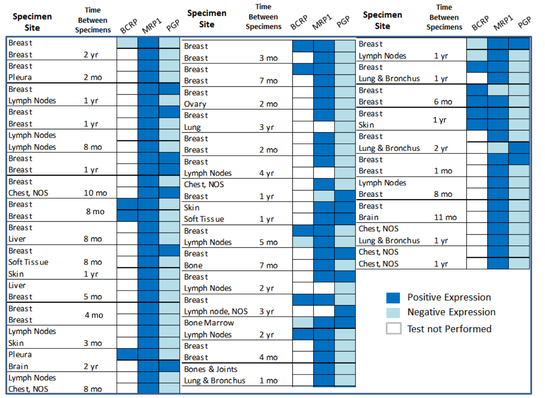


Figure 5. Selected Examples of Drug Pump Expression in patients profiled more than one time. MRP expression patterns remain consistently positive between specimen profiling, whereas, loss of expression in PGP (positive in first specimen, and negative in subsequent specimen) was observed in several patients, and to a lesser degree, gain of expression (negative in first specimen, and positive in subsequent specimen) was also observed.

Conclusions

- Triple negative breast cancer shows broad and overlapped expression patterns for drug efflux pumps. The identification of these biomarkers can be used to assess the likelihood treatment benefit for a variety of xenobiotic chemotherapy agents and stratify which treatment options are likely to provide the best chance for disease control in individual patients.
- MRP1 is overexpressed in the majority of TNBC, whereas PGP is overexpressed at a much lower frequency.
- Although PGP expression may be absent or lost during TNBC progression, MRP1 (and BCRP) expression are almost always retained.
- Further study is warranted to determine how transporter expression may impact clinical outcomes and contribute to chemotherapy resistance for particular chemotherapeutic agents.

References

1. Choi, Y.C. and A.-M. Yu, et al. (2014). "ABC Transporters in Multidrug Resistance and Pharmacokinetics, and Strategies for Drug Development.
2. Feldman, et al. (2014) ASCO. "Drug Efflux Pump Expression in 50,000 Molecularly Profiled Patients."

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