Genetic basis to an extraordinary response to Vinorelbine chemotherapy in metastatic triple negative breast cancer

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Case Report

Patients with metastatic triple negative breast cancer have a short median survival (17 months) (1). In these patients, first line chemotherapy results in less than 5% complete remissions while second or third line chemotherapy almost never results in sustained complete remission.

Two important classes of anticancer drugs utilized to treat metastatic breast cancer are: (a) vinca alkaloids such as vinorelbine and (b) taxanes such as paclitaxel (2). While these agents are effective, resistance develops quickly and patients with triple negative metastatic breast cancer have a median survival of less than 24 months after starting chemotherapy (1). The mechanism of action of the vinca alkaloids involves binding to tubulin heterodimers eventually causing dissolution of the mitotic spindle. In contrast, the taxanes bind in a different fashion reversibly to the microtubule polymer in a 1:1 stoichiometry relative to the tubulin heterodimer, within the lumen of the microtubule, enhancing the polymerization of tubulin, which is the opposite effect of vinca alkaloids (3,4,5).

Resistance to these agents has been investigated in cancer cell lines and xenograft cancer models. There are many possible mechanisms of resistance to tubulin modifying chemotherapy but the most prominent involves alteration in the tubulin proteins or microtubule-associated proteins (MAPs). Whereas increased βII-, βIII- and βIV-tubulin are associated with taxane resistance, decreased βIII-tubulin has been reported in vincarcisistant cell lines (6). Mutations affecting the expression of β-tubulin isotypes have been associated with altered sensitivity to these agents. Another line of defense against cytotoxic drugs includes regulatory proteins, in particular post-translational modifications (PTMs) of tubulin which can affect regulatory protein binding and alter sensitivity to tubulin modifying agents. In particular, the MAPs (tau, stathmin, MAP2 and MAP4) have been extensively investigated as triggers for cancer resistance (3, 6,7). However, mechanism of resistance in cell lines or xenograft models often are not easily translated into the clinic hampering the development of effective

Figure 1: Initial CT-scan showing metastases to the lung, spleen and right axillar and mediastinal LAD.
methods to overcome this resistance in clinical setting.

Paclitaxel-resistant cell lines contain “hypostable” microtubules in which the equilibrium between the dimer and polymer is shifted towards the dimer. These cells display increased resistance to polymer-binding drugs like paclitaxel, and increased sensitivity to vinca alkaloids. Thus, resistance to taxanes may be associated with sensitivity to vinca alkaloid (3).

A 58-year old female with stage 4 triple negative breast cancer (ER neg, PR neg, Her2 neg) with metastases to the right axillary, mediastinum, lung and spleen was treated with 3 different chemotherapy regimens (Gemcitabine plus capecitabine, paclitaxel and pegylated liposomal doxorubicin), and eventually failed all of them. She received fourth line vinorelbine chemotherapy for 2.5 years and had a complete response which has lasted more than 5 years with no maintenance chemotherapy. She appears to be cured.

In order to determine the genetic basis of this highly unusual result, her tumor DNA was analyzed in 2 different fashions: Foundation One testing which examines 206 genes involved in possible cancer therapy options and whole exome sequencing.

The whole exome sequencing done in collaboration with Dr Basik’s laboratory from the Lady Davis Institute for Medical Research (LDI) identified gene mutations that are thought to be deleterious; we meticulously identified each gene with a deleterious mutation and examined the function of the gene through database search/PubMed plus cross referencing the gene for possible interplay with the mitotic spindle or taxane/vinca alkaloid function/resistance, including survival and multidrug resistance-associated genes.

The following genetic abnormalities were identified by Foundation One testing: (a) amplification of CULA4, IRS-2, RAD21, RET and SDHD plus (b) a point mutation in NTRK2, TP53, ERBB4, FANCA, MLL2, NOTCH3, and SPEN. In addition to the point mutations identified in Foundation One testing, whole exome sequencing revealed several deleterious gene mutations in genes involved in the mitotic process which are of interest (KIF5A, PKHD1, CDC14a, c9orf114, CCAR1/CARP-1 and RPA2) (Table 1).

Examination of the amplified genes mentioned above indicates two important potential genes; IRS-2 and Rad21 involved in mitosis or involved in the response of tubulin-altering drugs such as vinorelbine and/or paclitaxel.
Overexpression of IRS-2 gene sensitizes MDA-MB-231 triple negative breast cancer cells to microtubule-disrupting agents such as vinorelbine but not taxol. Treatment with drugs that either stabilize or disrupt microtubules reveal that an intact microtubule cytoskeleton contributes to IRS-2 mediated activation of AKT leading to apoptosis. Indeed, breast cancer cells whose expression of IRS-2 was suppressed with short hairpin RNA had higher survival when exposed to vinca-alkoid compared to cells expressing IRS-2 (8,9,10). This differential sensitivity was not seen when these cells were exposed to Taxol. Thus, increased expression of IRS-2 may render breast cells hypersensitive to vinorelbine but not paclitaxel.

Rad21 is a component protein of cohesion involved in chromosomal segregation and errorfree DNA repair (11, 12, 13). Overexpression of Rad21 correlates with reduced sensitivity to non-vinca alkaloid chemotherapy in-vitro (survival of triple negative breast cells correlated with the level of Rad21 expression). In a population cohort of basal like breast cancer treated with chemotherapy, overexpression of Rad21 was associated with a worse overall survival (n = 30, p = 0.002) (11,). Beside IRS-2 and Rad21, the other amplified genes do not appear to have a direct involvement in mitosis or in binding to tubulin and its associated proteins.

The whole exome sequencing identified additional interesting mutated genes involved in mitosis: one of particular interest is KIF5A, a kinesin. Kinesins are involved in chromosomal and spindle movements during mitosis (14, 15, 16). Based on functional in-vitro work with triple negative breast cells and patient cohort studies of paclitaxel nonresponders, overexpression of KIF5A was associated with resistance to paclitaxel. There were no significant difference in survival in cells overexpressing the KIF5A compared to control cells upon exposure to doxorubicin or vincristine (14).

These results suggest that overexpression of KIF5A leads to a resistance to Paclitaxel but not vinca-alkaloids (14, 17).

Besides KIF5A, the other mutated genes do not appear to have a clinically relevant chemotherapy drug resistance involving taxane and vinca alkaloids.

Our genomic analysis has led to some gene alterations which could be responsible for this patient’s extraordinary response to the vinorelbine chemotherapy (IRS2, Rad21 and KIF5A). Analyzing DNA from an unusual responder to vinorelbine allows us to determine clinically relevant DNA alterations which would make us consider vinca alkaloids earlier in a patient’s treatment course.

Table 1. Genetic abnormalities were identified by A.

