Differentially regulated proteins in breast cancer chemotherapy

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Abstract

Intrinsic or acquired drug resistance of tumours is a major problem for successful therapy of breast cancer patients. The efficacy of doxorubicin, one of the most important and commonly used drugs in chemotherapy, can be severely compromised by a variety of unspecific mechanisms rendering tumours drug resistant. Little is known however, about the specific events taking place in response to doxorubicin treatment, which may repair doxorubicin-induced damage, leading to drug resistance.

Doxorubicin is a topoisomerase II poison, which interferes with topoisomerase II enzymes during DNA replication, resulting in DNA double-strand breaks. Topoisomerase II enzymes mediate the passage of DNA strands by introducing transient DNA breaks, and are essential for changes in DNA topology during replication. The DNA lesions induced by the combination of topoisomerase II and doxorubicin can be repaired by either non homologous end-joining or homologous recombination repair, as both pathways are specifically responsible for the repair of DNA double-strand breaks. The DNA-dependent protein kinase catalytic subunit in non homologous end-joining and Rad51 in homologous recombination repair are essential for each of these pathways. If it was possible to specifically target these proteins or other antagonistic mechanisms of doxorubicin-induced cell death, which may be activated in response to doxorubicin treatment, chemosensitivity of tumours could be restored and chemotherapy made more effective. Hence it was the purpose of this study to investigate proteome-wide changes in protein expression in response to drug treatment, as well as specifically analysing alterations in the protein levels of the DNA-dependent protein kinase catalytic subunit and Rad51.

Global changes in protein regulation of breast and breast cancer cells were investigated using mass spectrometric and electrophoretic analysis techniques. These experiments however, could not reproducibly identify any genuine drug-induced changes in protein levels, as only proteins of relatively high abundance could be analysed. Immunoblotting results however, showed that Rad51 was differentially regulated in a cell line- and drug dosage-dependent manner, while levels of the DNA-dependent protein kinase catalytic subunit remained largely unchanged. Furthermore, increased levels of topoisomerase II alpha protein were also detected. In addition, immunohistochemical analysis demonstrated that both Rad51 and the DNA-dependent protein kinase catalytic subunit could be independently overexpressed in breast tumours and therefore may represent potential targets for selectively enhancing chemosensitivity of breast cancers.

Abbreviations

ABC	ATP-binding cassette
AcN	Acetonitrile
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BSA	Bovine serum albumin
°C	Degrees Celsius
CAV2	Caveolin-2
CCND1	Cyclin D1 encoding gene
cDNA	Synthetic DNA, generated from RNA
CHAPS	3-[(3-Cholamidopropyl)dimethylamino]-1-propanesulphonate
CHCA	alpha-cyano-4-hydroxycinnamic acid
dCTP	deoxycytidine-triphosphate
DIGE	Differential gel electrophoresis
DMEM	Dulbecco's modified eagle's media
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleicacid
DNA-DSB	DNA double-strand break
DNA-PK	DNA-dependent protein kinase
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DTT	Dithiothreitol
2DGE	Two dimensional gel electrophoresis
EDTA	Ethylene diamine tetra-acetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ESI	Electro spray ionization
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum
Fig.	Figure
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulfonic acid]
HRR	Homologous recombination repair
HSP	Heat shock protein

ICAT	Isotope-coded affinity tagging
IEF	Isoelectric focusing
IPG	Immobilized pH gradient
kDa	Kilo Dalton
LC	Liquid chromatography
MALDI-MS	Matrix-assisted laser desorption ionisation mass spectrometry
MCE	Multi compartment electrophoresis
MCF12A	Mammary epithelial cell line
MCF7	Mammary epithelial carcinoma cell line
MDA MB 231	Mammary epithelial carcinoma cell line
MDR	Multidrug resistance
MDR1	Multidrug resistance gene
MEM	Eagle's minimal essential media
MRP	Multidrug resistance-associated protein
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MudPIT	Multidimensional protein identification technology
MXP	Mitoxantrone resistance associated protein
Mw	Molecular weight
MWCO	Molecular weight cut-off
m/z	Mass per charge
NHEJ	Non homologous end-joining
OC	Over-confluent
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBSE	Phosphate buffered saline plus EDTA
P-gp	P-glycoprotein
PIKK	Phosphatidylinositol 3-kinase-like kinase
PR	Progesterone receptor
p53	Tumour suppressor protein p53
R	resistant
RP-HPLC	Reversed-phase high performance liquid chromatography
RNase	Ribonuclease
RT	Room temperature

SCX	Strong cation exchange
SDS	Sodium dodecyl sulphate
SELDI-MS	Surface-enhanced laser desorption ionization mass spectrometry
T	Tyrosine
TBST	Tris buffered saline tween 20
TCA	Trichloroacetic acid
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFA	Trifluoroacetic acid
TGF	Transforming growth factor
Topo II alpha	topoisomerase II alpha
T 25, 75, 300	Filter top tissue culture flask of 25, 75, 300 cm^2
2-TCEP	Tris (2-carboxyethyl) phosphine
Xrcc4	X-ray repair cross-complementing protein 4)

List of tables

List of figures

Table of contents

Chapter Two: Materials and Methods

Chapter Seven: Summary and Discussion