CISPLATINUM SENSITIVITY OF BRCA1-MUTATED HCC1937 BREAST CANCER CELLS IS LINKED TO IMPAIRMENT OF NOTCH SIGNALING AND IS INCREASED BY GAMMA-SECRETASE INHIBITORS.

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Università degli Studi “Magna Graecia” di Catanzaro
CISPLATINUM

1965 Cisplatin (CDDP) was discovered by Barnett Rosenberg, who was using platinum electrodes to study the effect of electricity on bacterial growth. Cell division was dramatically reduced. Since cancer cells multiply rapidly, he reasoned that cisplatin might slow cancer growth.

1968 Was administered intraperitoneally to mice bearing a standard murine transplantable tumour of the day, sarcoma-180, and was shown to cause marked tumour regression.

1971 The first patient was treated with CDDP.

1978 Approved by the US Food and Drug Administration (FDA) for clinical use.
Mechanism of action

Covalently binds to DNA, forming DNA adducts.

The adducts cause distortions in DNA and activates various signal-transduction pathways; for example, those involved in DNA-damage recognition and repair, cell-cycle arrest, and programmed cell death/apoptosis.

Signal-transduction pathways that control growth, differentiation and stress responses have also been implicated.

The final cellular outcome is generally apoptotic cell death.
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Modena 18-19 novembre 2010

The resurgence of platinum-based cancer chemotherapy

Lloyd Kelland

Pablo Picasso Donna allo specchio, 1937
BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells.

CISPLATIN

DOXORUBICIN

PACLITAXEL
Breast cancer therapy: platinum standard.
Lugano N Imyanitov
Heredity in Clinical Practice 2003

Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo

Pier Francesco Tossone, 1,2,1 Maria Teresa Di Martino, 1,2,1 Monica Ventura, 1,2,1 Antonella Pietropaolillo, 1,2,1 Iole Cunicello, 1,2,1 Teresa Calmeri, 1,2,1 Alessandra Balotta, 1,2,1 Paola Neri, 1,2,1 Michele Caraglia, 1,2,1 Pier Giovanni Todini, 1,2
We have previously reported that HCC1937 BRCA1-defective human breast cancer cells are significantly more sensitive to CDDP than parental cells BRCA1-reconstituted (HCC1937/wtBRCA) by full-length cDNA transfection in vitro.

We analyzed the whole gene expression profile of HCC1937 and HCC1937/wtBRCA1 cells following in vitro exposure of tumour cells to CDDP, to identify the molecular bases of BRCA1-related differential sensitivity to the drug.
HCC1937 and HCC1937/wtBRCA1 have been exposed to CDDP at IC50 doses of 30 and 70μM, or at 60 and 140μM respectively, for 3, 12 and 24 hours.

Gene expression profiling was performed by Affymetrix technology using GeneArray 1.0 ST, expression arrays designed with approximately 26 probes for each of the 28,869 genes, spread across the full length of the gene.

Array data were analyzed using Gene Expression Console software, GeneSpring software and a Pathway-finder, Ingenuity Pathway Analysis (IPA).
Microarray analysis: results after 3 hours of CDDP exposure
This pathway includes critical cell cycle regulators of cell cycle progression such as Wee1, Cdc2 and ChK.

Wee1, Cdc2 and ChK are activated following DNA damage and lead suppression of cyclin activity, inducing mitotic arrest. This pathway was not affected in BRCA1-reconstituted cells.

Since G2/M damage check point is lost in HCC1937, probably these results are expression of a compensatory response to CDDP exposure.

Microarray analysis: results after 12 and 24 hours of CDDP exposure

Notch network down regulated in HCC1937 in vitro after 12 and 24 hours of CDDP exposure.
Rational targeted regulation by Numb over Notch signaling in breast cancer and associated with breast cancer and is associated with poor overall survival.

High-level Coexpression of JAG1 and Numb over Notch signaling in breast cancer and is associated with poor overall survival.


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ORIGINAL ARTICLE

ErbB-2 inhibition activates Notch-1 and sensitizes breast cancer cells

of ErbB2-Negative Human Breast Cancer Cells


Cross-talk between Notch and... Cancer Suggests Novel Therapeutic Approach

Paola Rizzo, Haixi Miao, Gwendolyn D'Souza, Clodia Osipo, Jieun Yun, Joaquina Mascarenhas, Debra Wyatt, Giovanni Antico, Lu Hao, Katharine Hao, Prabha Rajan, Chindo Hicks, Kalliopi Siziopikou, Suzanne Selvaggi, Amina Bashir, Deepali Bhandari, Adriano Marchese, Urban Lendahl, Jian-Zhong Qin, Debra A. Tonetti, Kathy Albain, Brian J. Nickoloff, and Lucio Miele

Western blotting CDDP 48 hours

<table>
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<th>microM doses</th>
<th>CNT</th>
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<th>10 microM</th>
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<td>N3ICD</td>
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<td>N1ICD</td>
<td>100</td>
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<td>83</td>
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</tr>
</tbody>
</table>

DMSO

CNT

Notch3ICD

Notch1ICD

HES1

β-actin

HCC1937
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MTT assay in HCC1937: GSI XII

IC 50: 20 microM
Western blotting after 48 hours of exposition

QRT-PCR GSI XII: Hes1 expression

Fold expression

DMSO  GSI XII 5uM  GSI XII 10uM

Notch3ICD  HES1

HCC1937

GSI XII

DMSO  5uM  10uM

Notch3ICD  Hes1  β-actin
Analysis of HCC1937 proliferation exposed to GSI XII 10 microM
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HCC1937 24 hours

100 80 60 40 20 0
% of growth

0 10 20 30 40 50 60
microM doses

IC 50 CDDP: 20 microM
IC 50 CDDP+GSI XII 10 microM: 0,5-1 microM

C.I.: 1.7
SYNERGIC EFFECT!

HCC1937 48 hours

HCC1937 72 hours
Work in progress…..

Silencing of Notch 1,2,3 and 4 in HCC1937

HCC1937 trasfections with N3ICD, N1ICD, ΔNNotch3

*In vitro and in vivo* chemosensitivity studies
Conclusions…

• Our findings indicate that high sensitivity of BRCA1-defective cells to CDDP exposure is related to depression of the DNA-damage repair machinery but also to down-modulation of survival pathways, as Notch pathway.

• CDDP induce loss of function of Notch3 signaling, a survival, ancestral pathway which BRCA1-defective cells could occur in cases of genotoxic stress.

• Our findings suggest that pharmacological inhibition of Notch3 may be a novel therapeutic approach in BRCA1-defective tumors, alone or in combination strategies.
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...THANK YOU...