Breast cancer cell growth/motility is influenced by metal compounds

Subjects: Cell Biology

Contributor: Claudio Luparello

Triple-negative breast cancer (TNBC) is a highly "aggressive" malignant neoplasm with limited treatment options due to the lack of expression of estrogen and progesterone receptors and HER2/neu. In search of novel molecules displaying anti-TNBC activities, the TNBC cell line MDA-MB231 was exposed to cadmium chloride and/or manganese chloride, and a biological characterization of the effect observed was performed. The data obtained demonstrate a cytotoxic effect exerted by cadmium chloride with drastic changes affecting gene expressions and production of reactive oxygen species. Conversely, manganese chloride was effective in increasing cell number and promoting cell invasive ability. Such effect was reverted by coexposure with cadmium chloride. Thus, metal compounds appear to be able to modulate the biological behavior of TNBC cells, although addressing them to different fates. The data obtained suggest that high environmental pollution with manganese chloride might increase the risk of breast tumorigenesis. On the other hand, the restraining modulatory property of cadmium chloride looks promising and deserves a more detailed mechanistic study aimed to the identification of possible molecular targets instrumental in inhibiting the expansion of malignant breast cancer.

Keywords: breast cancer cells ; MDA-MB231 ; manganese chloride ; cadmium chloride ; cell growth ; gene expression ; mitochondrial activity ; cell motility ; cell invasiveness

1. Introduction

[1]This article is related to the paper "Effect of Manganese Chloride and of Co-Treatment With Cadmium Chloride on the *In Vitro* Proliferative, Motile and Invasive Behavior of MDA-MB231 Breast Cancer Cells" that has been published by *Molecules*[2]. Triple-negative breast cancer (TNBC) is a highly "aggressive" malignant neoplasm with limited treatment options due to the lack of expression of estrogen and progesterone receptors and HER2/neu[3]. MDA-MB231 is a cell line derived from a pleural effusion of a TNBC of basal subtype and represents a suitable model system for *in vitro* investigation on this neoplastic histotype[4].

In search of novel molecules and/or treatment protocols displaying anti-TNBC activities, MDA-MB231 cells were exposed to different metal compounds. Literature data indicate that some of them can exert cytotoxic effects. For example, iron-manganese-doped sulfated zirconia nanoparticles were found to determine a concentration-dependent inhibitory effect on cell viability with prominent morphological changes attributable to cellular damage addressing them to death. Also, gallium and ruthenium complexes were proven effective on MDA-MB231 cells. In particular, the former ones were found to trigger cell cycle malfunctions and apoptosis by down-regulating AKT phosphorylation and activating caspases 3 and 7, and the latter ones were found active also in modifying the architecture of actin cytoskeleton and restraining the extracellular release of metalloproteinase-9 whose contribution to the invasive process is fundamental[5][6][7].

My research group have contributed to this field of research by investigating the effect of administration of different concentrations of cadmium chloride to MDA-MB231 cells, also comparing tumor cell behavior with that of HB2, a non-neoplastic immortalized cell line from human breast epithelium[8]. Our results demonstrated the cytotoxic effect of the compound with a 50% inhibitory concentration (IC_{50}) of 5 μM for cancer cells after 96 h-incubation. This concentration was uneffective in modifying the growth behavior of immortalized cells.

A biological characterization was performed on the effect of cadmium chloride administered at 96 h-IC_{50} to MDA-MB231 cells and the results are schematized as follows (see refs.[8][9][10][11][12][13]):

-Gene expression levels were up- or down-regulated as indicated below:

Gene	Protein product	up↑ down↓	Fold changes

HSPA5	**Endoplasmic reticulum chaperone BiP**	↓	**54.2**
HSPA8	**Heat shock cognate 71 kDa protein**	↓	**4.9**
HSPB1	**Heat shock protein beta-1**	↑	**8.7**
HSPD1	**60 kDa heat shock protein, mitochondrial**	↓	**2**
HSP90AB1	**Heat shock protein HSP 90-beta**	↓	**2.57**
TRAP1	**Heat shock protein 75 kDa, mitochondrial**	↓	**9.5**
MT1A	**Metallothionein-1A**	↑	**2.34**
MT1F	**Metallothionein-1F**	↑	**3.65**
MT1G	**Metallothionein-1G**	↓	**18.8**
BCL2	**Bcl-2**	↓	**53**
WAF1	**Cyclin-dependent kinase inhibitor 1**	↑	**10.4**
DAPK	**Death-associated protein kinase-1**	↑	**55**
RIPK1	**Receptor-interacting protein 1**	↑	**undetectable in control**
CASP1	**Caspase-1**	↑	**106**
CASP2	**Caspase-2**	↑	**3**
CASP6	**Caspase-6**	↑	**31.3**
CASP7	**Caspase-7**	↑	**15**
CASP8	**Caspase-8**	↑	**9.25**
CASP9	**Caspase-9**	↑	**4.7**
MAPK14	**Mitogen-activated protein kinase p38 alpha**	↓	**8**
MAPK11	**Mitogen-activated protein kinase p38 beta**	↓	**4**
MAPK12	**Mitogen-activated protein kinase p38 gamma**	↑	**7**
COX2	**Cytochrome c oxidase subunit 2**	↓	**3**
COX4	**Cytochrome c oxidase subunit 4**	↓	**1.9**
AEG1	**Astrocyte elevated gene-1 protein**	↓	**8.5**
PLP2	**Proteolipid protein 2**	↑	**2**
FOS	**Proto-oncogene c-Fos**	↓	**3.2**
JUN	**Proto-oncogene c-Jun**	↓	**3.5**

-At protein accumulation level, a decrease was found for mitochondrial hsp60, MAP kinase p38 gamma, astrocyte elevated gene 1-protein and cytochrome c oxidase subunits 2 and 4, whereas an increase was observed for cytosolic hsp60, hsp70, hsc71 and MAP kinase p38 beta.

-The nuclear import of astrocyte elevated gene 1-protein and NF-kB p65 was down-regulated.

-Mitochondrial respiratory activity increased and a massive production of reactive oxygen species was found, although the mitochondrial transmembrane potential was not affected.

A second set of experiments was aimed to examine the dose–response effect of different concentrations of another metal compound, i.e. manganese chloride, on MDA-M231 cell viability and growth. Also in this case tumor cell behavior was compared with that of immortalized HB2 cells. Such evaluation was supplemented by the analysis of tumor cell motility and invasiveness *in vitro*, the latter being quantitated by specific parameters such as invasion index (I.I.) and relative invasion index (R.I.I.)[2].

Exposure to 1, 5, 10, and 50 µM manganese chloride for 96 h resulted in an increase of cell population up to about 32, 52, 47, and 32% *vs.* untreated controls, respectively. Conversely, exposure to 100 µM manganese chloride for 96 h determined a prominent decrease of cell number. The viability and proliferative behavior of HB2 cells was not significantly

modified by the presence of manganese chloride in the culture medium, except for the highest concentrations which determined a reduction of cell number.

2 .Experiment

Boyden chamber assays were then performed to evaluate tumor cell motility and invasiveness after exposure to 5 µM concentration of manganese chloride for 96 h, which in the proliferation assays induced the more elevated increase of the cell population. In particular, the experiments were performed using “blind well” chamber (top well = 800 µL, bottom well = 200 µL; purchased from Neuro-Probe, Cabin John/MD, USA). Polyvinylpyrrolidone-free polycarbonate filters with an 8-µM pore diameter and 50 mm^2 exposed area (purchased from Nucleopore, Pleasanton/CA, USA) were allocated in the chamber and coated with either type I collagen for chemoinvasion assays, or matrigel, a reconstituted 3D basement membrane matrix from Engelbreth–Holm–Swarm murine sarcoma, for chemonvasion assays. Under this experimental condition, following exposure to manganese chloride the invasive ability of MDA-MB231 cells was found enhanced with an R.I.I. of 2.78.

In a third set of experiments, we evaluted the proliferative and invasive behavior of breast cancer cells following coexposure to 5 µM of both cadmium and manganese chloride for 96 h. Under this experimental condition, the results obtained demonstrated a reversion of the growth-promoting effect observed after exposure to manganese chloride only. Similarly, the R.I.I. was found to diminish from 2.78 down to 1. The explanation of this finding may rely, at least in part, upon the known competition of manganese and cadmium for the same transmembrane transport systems, and the impairment of activity of mitochondrial manganese superoxide dismutase due to the alterations induced by cadmium on mitochondrial respiration[13][14]

In conclusion, metal compounds appear to be able to modulate the biological behavior of TNBC cells, such as MDA-MB231, although addressing them to different fates. Although caution must be exercised in extrapolation of *in vitro* results to the *in vivo* situation, the data obtained suggest that high environmental pollution with manganese chloride might increase the risk of breast tumorigenesis in exposed humans by promoting the growth and spreading of subpopulations of malignant TNBC cells. On the other hand, the restraining modulatory property of cadmium chloride against the proliferation and invasiveness of manganese-treated MDA-MB231 cells looks promising and deserves a thorough study of the involved intracellular mechanisms and pathways aimed to the identification of possible molecular targets instrumental in inhibiting the expansion of malignant breast cancer.

References

1. C Luparello; M A Birch; J A Gallagher; W J Burtis; Clonal heterogeneity of the growth and invasive response of a huma n breast carcinoma cell line to parathyroid hormone-related peptide fragments. *Carcinogenesis* **1997**, *18*, 23-29, 10.10 93/carcin/18.1.23.
2. Claudio Luparello; Effect of Manganese Chloride and of Cotreatment with Cadmium Chloride on the In Vitro Proliferativ e, Motile and Invasive Behavior of MDA-MB231 Breast Cancer Cells. *Molecules* **2019**, *24*, 1205, 10.3390/molecules24 071205 .
3. Adrienne G. Waks; Eric P. Winer; Breast Cancer Treatment. *JAMA* **2019**, *321*, 288-300, 10.1001/jama.2018.19323.
4. Kathryn J. Chavez; Sireesha V. Garimella; Stanley Lipkowitz; Triple Negative Breast Cancer Cell Lines: One Tool in the Search for Better Treatment of Triple Negative Breast Cancer. *Breast Disease* **2011**, *32*, 35-48, 10.3233/BD-2010-030 7.
5. Mohamed Qasim Al-Fahdawi; Abdullah Rasedee; Mothanna Sadiq Al-Qubaisi; Fatah H Alhassan; Rozita Rosli; Moham ed Ezzat El Zowalaty; Seïf-Eddine Naâdja; Thomas J Webster; Yun Hin Taufiq-Yap; Rasedee Abdullah; et al. Cytotoxici ty and physicochemical characterization of iron–manganese-doped sulfated zirconia nanoparticles. *International Journ al of Nanomedicine* **2015**, *10*, 5739-5750, 10.2147/IJN.S82586.
6. Charles Saby; Gilane Mohamed Sabry; Rasha Elsherif Hassan; Abdelfattah Badawi; Pierre Jeannesson; Ahmed Mohse n; Philippe Collery; Hamid Morjani; Didier Desmaële; Biological study of the effect of water soluble [N-(2-hydroxybenzy l)-l-aspartato] gallium complexes on breast carcinoma and fibrosarcoma cells. *JBIC Journal of Biological Inorganic Che mistry* **2016**, *21*, 837-849, 10.1007/s00775-016-1382-7.
7. Cecília P. Popolin; João P. B. Reis; Amanda B. Becceneri; Angélica E. Graminha; Márcio A. P. Almeida; Rodrigo S. Corr ea; Legna A. Colina-Vegas; Javier Ellena; Alzir A. Batista; Marcia Regina Cominetti; et al. Cytotoxicity and anti-tumor ef fects of new ruthenium complexes on triple negative breast cancer cells. *PLoS ONE* **2017**, *12*, e0183275, 10.1371/jour nal.pone.0183275.

8. Rosalia Sirchia; Alessandra Longo; Claudio Luparello; Cadmium regulation of apoptotic and stress response genes in t umoral and immortalized epithelial cells of the human breast. *Biochimie* **2008**, *90*, 1578-1590, 10.1016/j.biochi.2008.0 6.011.
9. Giuseppe Cannino; Elisa Ferruggia; Claudio Luparello; Anna Maria Rinaldi; Effects of cadmium chloride on some mitoc hondria-related activity and gene expression of human MDA-MB231 breast tumor cells. *Journal of Inorganic Biochemist ry* **2008**, *102*, 1668-1676, 10.1016/j.jinorgbio.2008.04.002.
10. Caterina Casano; Maria Agnello; Rosalia Sirchia; Claudio Luparello; Cadmium effects on p38/MAPK isoforms in MDA-MB231 breast cancer cells. *BioMetals* **2009**, *23*, 83-92, 10.1007/s10534-009-9268-6.
11. Claudio Luparello; Alessandra Longo; Marco Vetrano; Exposure to cadmium chloride influences astrocyte-elevated gen e-1 (AEG-1) expression in MDA-MB231 human breast cancer cells. *Biochimie* **2012**, *94*, 207-213, 10.1016/j.biochi.201 1.10.009.
12. Alessandra Longo; Mariangela Librizzi; Claudio Luparello; Effect of transfection with PLP2 antisense oligonucleotides o n gene expression of cadmium-treated MDA-MB231 breast cancer cells.. *Analytical and Bioanalytical Chemistry* **2012**, *405*, 1893-1901, 10.1007/s00216-012-6182-5.
13. Claudio Luparello; Rosalia Sirchia, Laura Paci; Vitale Miceli; Rosaria Vella; Rosaria Scudiero; Francesca Trinchella. Re sponse to Cadmium Stress by Neoplastic and Immortalized Human Breast Cells: Evidence for Different Modulation of Gene Expression. In: Trends in Signal Transduction Research; Jennifer N Meyers, Eds.; Nova Science Publ.: Happaug e/NY (USA), 2007; pp. 87-113.
14. Seiichiro Himeno; Takahiro Yanagiya; Hitomi Fujishiro; The role of zinc transporters in cadmium and manganese transp ort in mammalian cells. *Biochimie* **2009**, *91*, 1218-1222, 10.1016/j.biochi.2009.04.002.
