Synergistic effects of chemotherapeutic agents and polyphenolic artichoke extracts in breast cancer cells and the potential immunomodulatory activity of these dietary polyphenols

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Many epidemiological studies have shown that diet particularly rich in polyphenols has antitumor, chemopreventive and immunomodulatory activities. The growing interest for dietary phytochemicals had led to a renewed attention on artichoke, a food rich in polyphenols. We demonstrated the antiproliferative activity of polyphenolic extracts from the edible part of artichoke (AEs) on many cancer cell lines derived from different histological origin (1-3). Focusing on breast cancer we demonstrated that AEs induce apoptosis and decrease the invasive potential of human breast cancer cell lines (4). Furthermore our group has showed that chronic and sub-lethal doses of AE treatment inhibits breast cancer cell growth via the induction of premature senescence through epigenetic and ROS-mediated mechanisms (3).
Recently data from literature demonstrated that combined treatment of natural polyphenols and chemotherapeutic agents are more effective with respect to the agent alone in controlling the growth of cancer cells.
In order to investigate the potential antitumor synergistic properties of AEs in combination with the most active cancer drugs (taxols and anthracyclines) we used as experimental models, two breast cancer cell lines, MCF-7 and MDA-MB 231, respectively luminal A and triple negative subtypes.
Now we demonstrate a synergistic cytotoxicity when cells were treated with a low dose of paclitaxel (PTX) or adriamycin (ADR) in association with a low dose of AEs compared with the antitumor activity of single agents. Combined administration significantly reduces cell proliferation in AEs dose-dependent manner without inducing an apoptotic and autophagic cell death. In the same experimental setting, cell motility and cellular clonogenic ability are not affected. The combined PTX and AEs treatment enhances breast cancer cell sensitivity to PTX by down-regulating the expression of Flap endonuclease 1 (FEN1), known to promote DNA replication and repair. Our findings, consistent with literature data, show that down-regulation of FEN1 expression can effectively inhibit the proliferation of breast cancer cells. A reduced FEN1 level leads to the accumulation of un-repaired DNA intermediates resulting in DNA double strand breaks, as demonstrated by the increasing of γH_2AX, a sensitive marker for DNA damage.
In conclusion based on the roles of FEN1 in DNA repair, we suggest that AE/chemotherapy, through the inhibition of FEN1 and generation of DNA damage, sensitizes cancer cells to chemotherapeutic treatment.
In the context of promising immune-based approaches (5,6) we are moving at studying the potential immunomodulatory role of AEs by treating peripheral blood lymphocytes (PBMC) of healthy donors and cancer patients with this natural compound and evaluating its effect on PBMC phenotype pattern and functional activity also in terms of cytokine production.

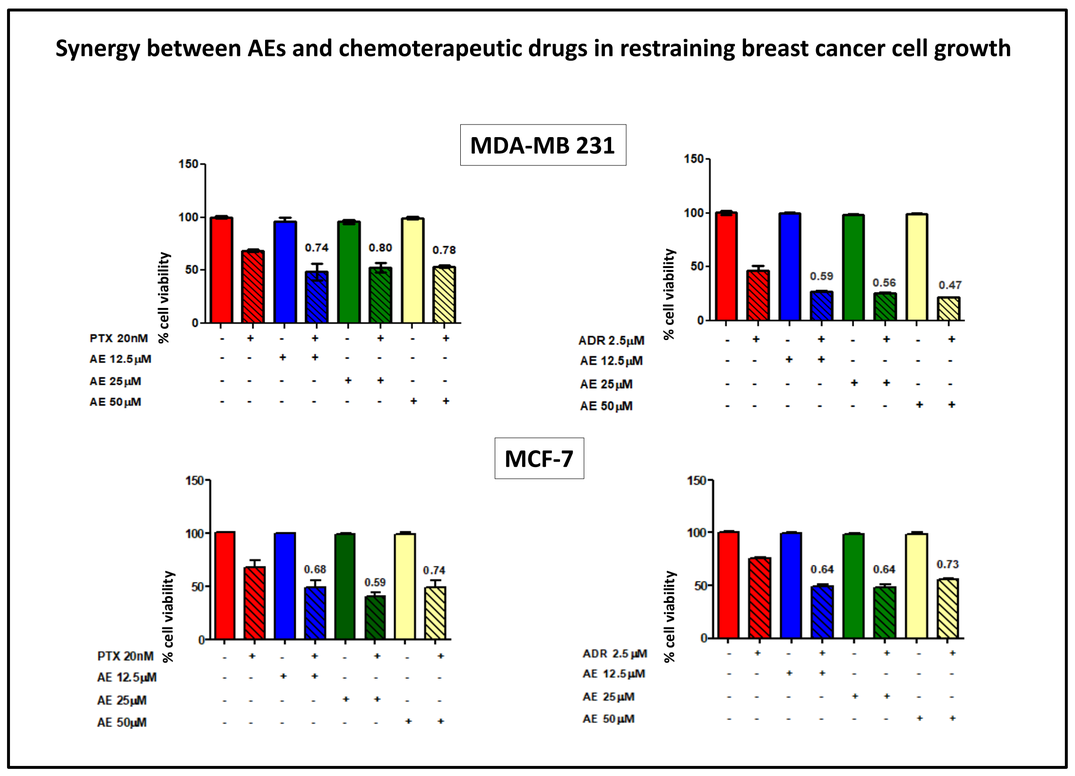


Fig.1 Two breast cancer cell lines, MDA-MB 231 and MCF-7 were treated with 20 nM Paclitaxel (PTX) or 2.5 µM Adriamycin (ADR) together with increasing doses of AEs . After 24 h cell viability was assessed and the combination index (CI) was calculated as reported in the histograms. Synergy is characterized by CI ≤0.8

RESULTS

1) The combined treatment, AEs plus PTX or AEs plus ADR, yielded a decrease in cell viability attributable to a chemosensitizing effect of AEs to these chemoterapeutic agents. Such a treatment enhances cytotoxicity in a synergistic manner, as demonstrated by CI values ≤ 0.8 (Fig.1).
2) We evaluated the molecular signs of apoptotic and autophagic cell death. The amount of the cleavage product of PARP, a well known marker of apoptosis, is slightly increased in the cells treated with 25 µM AEs plus PTX compared to PTX alone (Fig.2). By measuring the expression of LC3 as autophagic hallmark, we found that the protein modulation was not statistically relevant (data no shown). These results demonstrate that both apoptotic and autophagic cell deaths are scarsely implicated in the synergistic effect of the combined treatment.
3) In order to achieve a better comprehension of the synergistic effect, we investigated the clonogenic (Fig.3) and migration ability (Fig.4) of combined treated breast cancer cells. The minor differences detected were not sufficient to explain the synergistic response in our experimental setting .
4) To identify the crucial mechanism implicated in the synergistic effect of AEs/PTX combined treatment, we detected the rate of cell proliferation in MDA-MB 231 cells. AEs/PTX strongly inhibited ^{3}H-thymidine cellular incorporation in a AE dose dependent manner (Fig.5) and focused on a relevant molecular role of Flap endonuclease 1 (FEN1). As detailed in Fig.6a/b 25 µM Aes, when employed in combination with PTX, triggered down-regulation of FEN1 expression. In the same experimental setup we demonstrated an evident decrease of p-ERK1-2 correlated to FEN1 modulation as demonstrated by the effect of p-ERK inhibitor UO126 (Fig.6c).
5) In order to evaluate DNA damage level related to FEN1 down-regulation, an endonuclease known to promote DNA repair, we detected a marked phosphorylation level of γH_2AX, a biomarker for DNA double-strand breaks (Fig.7).

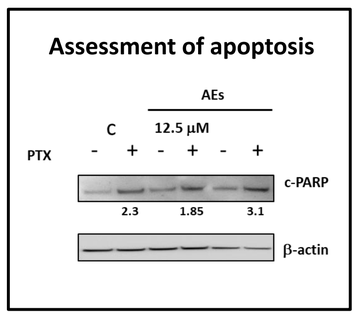


Fig.2 Analysis of cell apoptosis by evaluation of c-PARP in AE/PTX treated MDA-MB 231 cells.

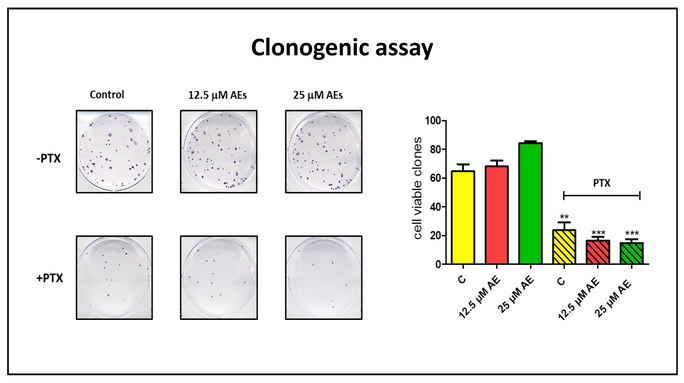


Fig.3 Colony forming ability. MCF-7 cells were exposed to combined treatment and the number of clones was evaluated compared to PTX alone.

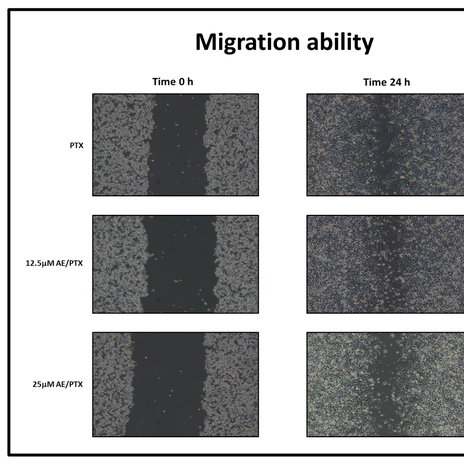


Fig.4 Effect of combined treatment on migration ability of MDA-MB 231 cells.

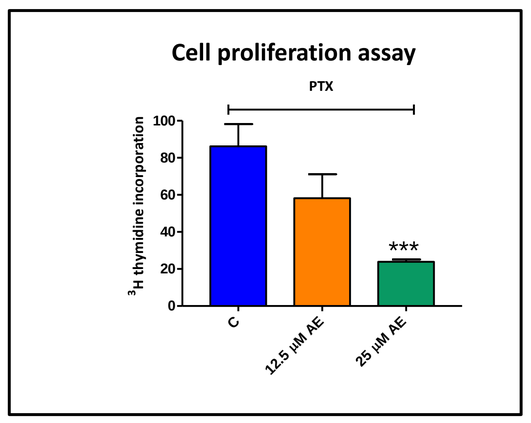
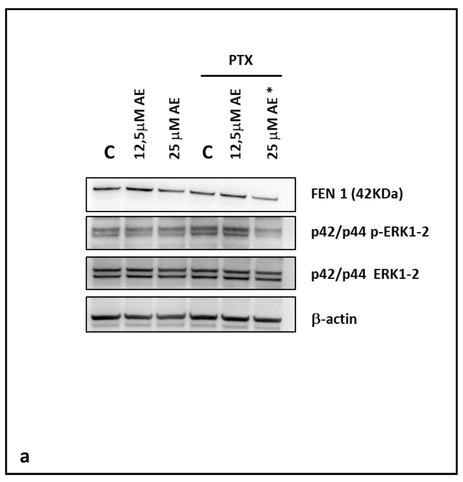
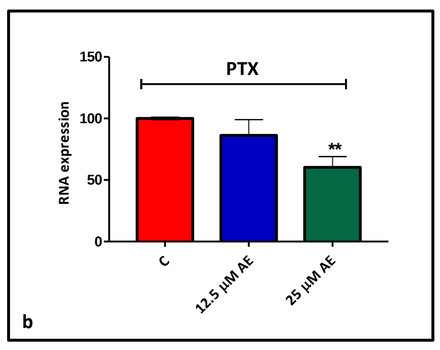


Fig.5 Assessment of cell proliferation by ^{3}H-thymidine incorporation in MDA-MB 231 cells synergistically treated.





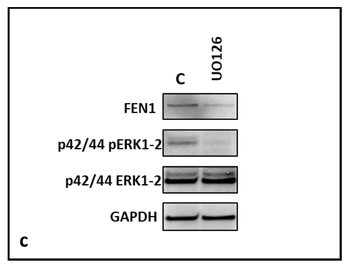


Fig.6 Down-regulation of FEN1 expression and hypo-phosphorylation of ERK1-2. **a**) Analysis of modulation of FEN1 and p-ERK1-2 proteins, **b**) FEN1 RNA expression by RT-PCR analysis, **c**) down-regulation of FEN1 mediated by UO126 (p-ERK 1-2 inhibitor).

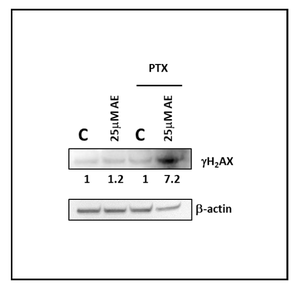


Fig.7 DNA damage assessment. Analysis of γH_2AX expression as a biomarker for DNA double strand breaks in combined treated MDA-MB 231 cells.

CONCLUSION

Altogether our data demonstrated that the simultaneous treatment of polyphenolic extracts of artichoke (AEs) and low doses of chemoterapeutic agents potentiates the effect of antitumor therapy in breast cancer cells. Therefore we envisage a combined AEs and PTX/ADR treatment as a novel strategy able to reduce the dose of chemotherapeutic drugs, to minimize toxicity and side effects of conventional therapy in breast cancer patients. In the light of innovative and promising immune-based therapeutic strategies, we are studying the potential immunomodulatory role of AEs. From a therapeutical point of view it will be a challenge to deeply evaluate the role of AEs as anticancer and immunomodulatory agent to be used in clinical setting. Dietary polyphenols could be exploited to improve the efficacy of novel cancer treatment such as immune checkpoint blockade, a new reality in oncology.

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