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Mechanisms of chemotherapy-induced changes in apoptosis-related proteins

High-dose cytarabine in combination with anthracyclines represents a standard of acute myeloid leukemia (AML) treatment. However, this intensive therapy is not suitable for all AML subtypes, in particular for older patients. Alternative treatment strategies frequently use epigenetic drugs, which induce changes in methylation and acetylation profile of nucleic acids and proteins. We investigate mechanisms of action of both standard chemotherapy drugs, e.g. cytarabine (ara-C), idarubicin, actinomycin D or all-trans retinoic acid (ATRA), and epigenetic drugs, 5-aza-2'-deoxycytidine (decitabine, DAC) and suberoylanilid hydroxamic acid (SAHA, Vorinostat). In addition, we also study the effects of specific inhibitors of nuclear export (selinexor, eltanexor) or of agents affecting the interaction between the tumor suppressor p53 and its regulatory protein, mdm2 (Nutlin3A, RITA). Deregulation of programmed cell death (apoptosis) is the most common feature of tumors. Chemotherapy targets various apoptotic pathways to restore normal apoptotic process and thereby induces elimination of tumor cells. However, this type of treatment is not tumor-specific and partially also affects non-tumor cells. Drug combinations are useful to achieve maximal effect on tumor cells and, concurrently, to minimize damage of normal cells. We focus on study of proteins related to apoptosis, mainly the tumor suppressor p53, its target proteins (p21, Puma, and Noxa), proapoptotic and anti-apoptotic proteins of Bcl-2 family, and proteins belonging to the group of inhibitors of apoptosis (IAP).

We published several papers describing the effect of decitabine and SAHA, and of their combination with ATRA, on apoptotic process in leukemia cell lines as well as in normal peripheral blood mononuclear cells (PBMC). Following results have been presented there:
1) Decitabine in high concentrations (8 μM) induces p53-dependent apoptosis in both leukemia cell line CML-T1 and PBMC of healthy donors. The apoptosis is further augmented by SAHA (4 μM) [1]. Simultaneously, reactive oxygen species (ROS) induced by decitabine and SAHA co-treatment also contribute to cell death [2].
2) Low concentrations of decitabine and SAHA (both 1 μM) do not cause substantial changes in normal PBMC. However, this combination induces p53-dependent apoptosis in leukemia cell lines possessing the wild-type p53. Apoptosis caused by ROS induction, decreased level of anti-apoptotic protein Bcl-2, and activation of proapoptotic protein BAX is launched by this combination in p53-null cells [3].
3) In the p53-mutated cells, suppressed proliferation after decitabine treatment was found to be mediated also by incomplete localization of Survivin in kinetochores of mitotic cells. Survivin is the smallest member of IAP family, which also regulates binding of mitotic spindle fibers into the kinetochores. Its aberrant localization in decitabine-treated cells indicates a connection between the two functions of the Survivin [4].

Analysis of cytarabine (Ara-C) effect on leukemia cell lines confimed that a single dose of Ara-C induces cell cycle arrest in the S-phase. After 24 hours, the S-phase arrest is followed by the recovery of the treated cells and the cell viability is not substantially affected.
In our recent work [5] we demonstrated that the resistence to single-dose cytarabine treatment is accompanied with Survivin overexpression in interphase cells. The Survivin induction was detected in all leukemia cell lines despite the presence of various mutations characteristics for AML, i.e. *NPM1*, *FLT3-ITD*, *DNMT3A* or *TP53* mutations. Increased Survivin levels were inhibited by concurrent idarubicin treatment in majority of the tested cell lines (Fig.1). Alternatively, a specific Survivin inhibitor, YM-155, decreases survivin level in the cell lines resistant to idarubicin. Contrary to unique cytarabine activity, effects of idarubicin/YM-155 are more variable and likely reflect the heterogeneity of AML.

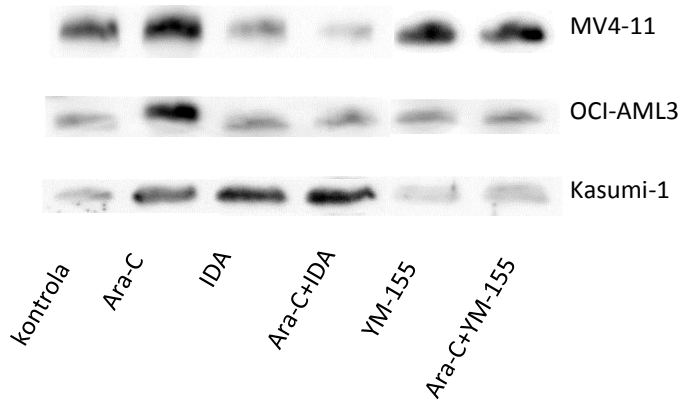

Fig. 1: Survivin levels in various AML-derived cell lines after treatment with 5 μM cytarabine (Ara-C), 100 nM idarubicine (IDA), 200 nM YM-155 or their combinations.

References:

[1] Brodska B, Otevrelova P, Holoubek A (2011) Decitabine-induced apoptosis is derived by Puma and Noxa induction in chronic myeloid leukemia cell line as well as in PBL and is potentiated by SAHA. *Mol.Cell.Biochem.* 350(1-2):71-80
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[5] Otevřelová P and Brodská B (2021) Chemotherapy-Induced Survivin Regulation in Acute Myeloid Leukemia Cells. *Applied Sciences* 11(1):460