REVIEW

The Retinoid Induced Pancreatic Cancer Redifferentiation-Apoptosis Sequence and the Mitochondria: A Suggested Obligatory Sequence of Events

Tarek H El-Metwally[1], Parvis M Pour[2]

[1]Department of Medical Biochemistry, Faculty of Medicine, Assiut University. Assiut, Egypt.
[2]Department of Pathology and Molecular Biology, University of Nebraska Medical Center. Omaha, NE, USA

Summary

Retinoic acid induces redifferentiation and apoptosis in pancreatic adenocarcinoma cell lines. Redifferentiation includes early reversion into aerobic metabolism as reflected by an increase of mitochondrial activity and mass with normal membrane potential and terminal ductal cell differentiation. Cells in such a state either attempt to correct their DNA abnormalities or commit suicide by apoptosis. In some cell systems, such as pancreatic ductal cells, the stem cells show potential to transdifferentiate into functional normal endocrine cell type. However, since it is impossible to correct a highly corrupted genome, cells eventually succumb to apoptosis. Mitochondrial changes appear to be the enforcing factor for this process. The Transformation - Normalizing-redifferentiation - Apoptosis sequence has been shown by several studies, utilizing various cell types, apoptotic inducers, biomarkers and time frames. Although some studies have shown concomitant apoptosis and redifferentiation, others have reported apoptosis without prior redifferentiation. However, utilizing the appropriate time frame and the markers of earlier mitochondrial changes, one would detect a scenario similar to the retinoid model. This situation can be achieved by delaying apoptosis or reducing the inducer concentration in such systems. The final physiological fate of a normal terminally differentiated cells is apoptosis. Similarly, it is suggested that a degree of normalizing redifferentiation of transformed cells might be expected prior to apoptosis. The former seemed obligatory at least in the retinoid-pancreatic model.

The progress in differentiation depends on an increase in the ratio between mitochondrial differentiation-promoting activity and nuclear differentiation-preventing activity. This ratio is low in embryonic and stem cells, due to low mitochondrial content, but it increases by a rate of mitochondrial multiplication that is larger than a doubling of mitochondrial content per cell cycle. The rate of mitochondrial multiplication, therefore, determines the progress in differentiation and subsequent apoptosis as a physiological fate. This rate is modifiable by extracellular signals and cellular defects. Mutations and cytoskeletal changes are likely to decrease the rate to the degree that differentiation is arrested with the ensuing neoplastic growth. Agents used in chemical therapy and ionizing radiation overcome this arrest by preferentially targeting the cell cycle. The
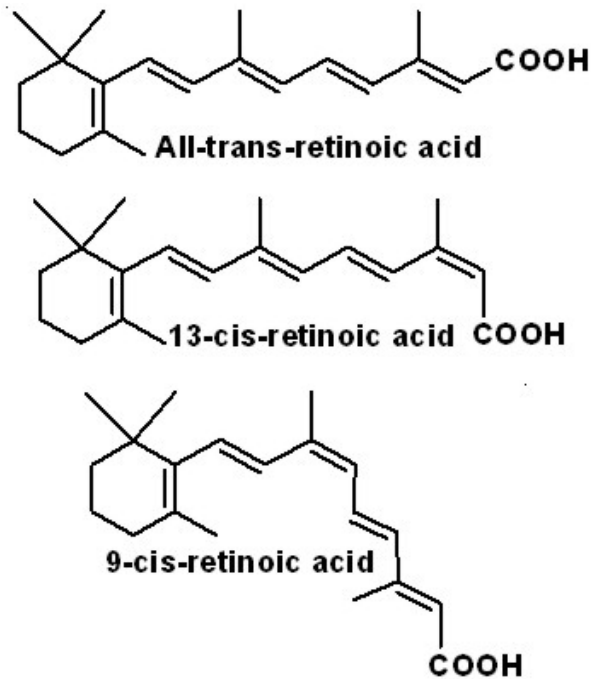
Figure 1. Chemical structures of the three utilized natural isomers of retinoic acid.

mitochondria multiply during the transitory cell cycle inhibition, resulting in increased differentiation-promoting activity. The finding of an increase in mitochondrial mass and an induction of differentiation prior to the release of cytochrome c and apoptosis points to the integration of the initial molecular pathways of differentiation and apoptosis [1]. Differentiation is a short-term program of biogenesis responsible for the rapid changes in the bioenergetic phenotype of mitochondria. In contrast, proliferation is a long-term program responsible for the decrease in mitochondrial mass in the cell. Moreover, some tissues, such as the fetal liver, have many phenotypic manifestations in common with highly glycolytic tumor cells [2]. The differentiation-dedifferentiation process during transformation is a gradual loss of tissue-specific characteristics from benign, well-differentiated, moderately-differentiated, poorly-differentiated to undifferentiated states. Likewise, dedifferentiation-redifferentiation process should not be looked at as an on/off process. But rather, it is a stepwise retrograde acquisition of the lost biochemical and phenotypic properties. The least of such biochemical redifferentiation is the reversion into the aerobic metabolism featured as a

normal mitochondrial membrane potential ($\Delta\Psi_m$) and increased mitochondrial mass and activity, as observed in the retinoid-induced pancreatic cancer redifferentiation-apoptosis sequence [3, 4, 5, 6, 7].

The importance of the mitochondria in cell life and death is clear from being a major target for the critical balance between pro- and anti-apoptotic effectors particularly among the Bcl2 family. In principle, biochemically, a cancer cell is a cancer cell even in the submicroscopic stage. Tumor progression is usually associated with a partial or a complete loss of morphological and biochemical features of the original tissue by the process of dedifferentiation. To a certain extent, carcinomas preserve differentiation markers of normal tissue, and hemoblastoses precisely reflect the direction and differentiation level of their precursor cells. However, both carcinomas and hemoblastoses retain the ability to differentiate [7]. In cancer cells the activities, amounts, and pattern of key enzymes is stringently linked with transformation and progression. In parallel, with the rise in tumor growth rates there is an increase in the activities of the key glycolytic enzymes in an aerobic manner (conversion of glucose to lactate); glucokinase/hexokinase, 6-phospho-fructokinase and pyruvate kinase [8]. Accordingly, the glycolytic shift of cancer cell metabolism is an integral part of the transformation process rather than an adaptive measure [9].

In our retinoic acid model (Figure 1), using human pancreatic adenocarcinoma cells, we noticed that the direct MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) cell proliferation assay greatly masked the antiproliferative effect of retinoic acid compared to cell count, total proteins or ^{3}H-thymidine incorporation [10]. Since early transformation is associated with aerobic glycolysis and a reduction of mitochondrial activity and mass, it was hypothesized that a minimum degree of redifferentiation (i.e., malignant Transformation - Normalizing-redifferentiation - Apoptosis sequence, contrasting, normal-transforming-

dedifferentiation-immortalization sequence) is required for cancer cell to succumb to apoptosis. Many would oppose this hypothesis, claiming a full-scale tissue-specific redifferentiation as a prerequisite. However, what seems to be of profound importance is the reversion into aerobic metabolism through mitochondria. The degree of aerobic redifferentiation could be the least of the requirements for the induction of apoptosis that would accommodate any kind of apoptotic inducer or cell model. As a result, early features of redifferentiation that embrace such a scenario depends on the timeframe of the apoptotic induction, the type and concentration of the apoptotic inducer and the cell type used. Therefore, the observed induction of reductive MTT activity, when it was normalized to the number of cells or total protein content, was expected because of the induction of the mitochondrial activity and mass correlating redifferentiation. Early retinoic acid-induced redifferentiation was associated with a several-fold increased induction of MTT, especially when apoptotic concentrations of retinoic acid were used (Figure 2). This would reflect the induction of mitochondrial dehydrogenase activities. Untreated pancreatic cancer cells, growing in wells along the periphery of the tissue culture plates, showed reduced glucose utilization, lactate production and proliferation compared to cells within the internal wells (unpublished observations). The former cells possibly received more oxygen from the circulating incubation gas than cells in the internal wells. This suggests that the mere aeration of cancer cells could be antiproliferative and redifferentiating. This contrasts with the effect of hypoxia that has an adverse effect on the prognosis of cancer in vivo. As expected, increasing the oxygen concentration in cancers at the tissue and cellular levels reduced the hypoxia-induced cancer cell aggression and expression of angiogenic genes, a good prognostic biomarker [11, 12]. It follows that benign and well-differentiated cancers are well-vascularized and aeriated. Although the cells of these lesions, as well as cells that appear normal, are already

glycolytic, they do not have metastatic potential [13, 14, 15]. Transformation of colonic epithelial cells is characterized by decreased mitochondrial activity, increased $\Delta\Psi_m$, and disrupted proliferation-apoptosis equilibrium. Decreased mitochondrial gene expression is an early marker of colon cancer and tumorigenesis, thereby implicating alterations in mitochondrial function as an early event in the transformation of colonic epithelial cells, regardless of the etiology. Moreover, an intact $\Delta\Psi_m$ is essential for growth arrest and apoptosis induced by butyrate [16, 17, 18]. Similar to the observed induction of MTT reducing activity, retinoic acid promoted nitroblue tetrazolium-associated functional cell differentiation in prostate cancer PC-3 cells with a re-activation of the tumor suppressor p75 neurotrophin receptor [19]. Additionally, the natural isoflavone genistein was antiproliferative on MCF-7, human breast tumor; Jurkat cells, human T-cell leukemia; L-929, mouse transformed fibroblasts cell lines *in vitro* with a G2/M cell-cycle arrest and an increase in cell volume and in mitochondrial number and/or activity. Consequently, the significant influence of genistein on mitochondrial number and/or function resulted in a sequential increase in MTT reduction to formazan per cell [20].
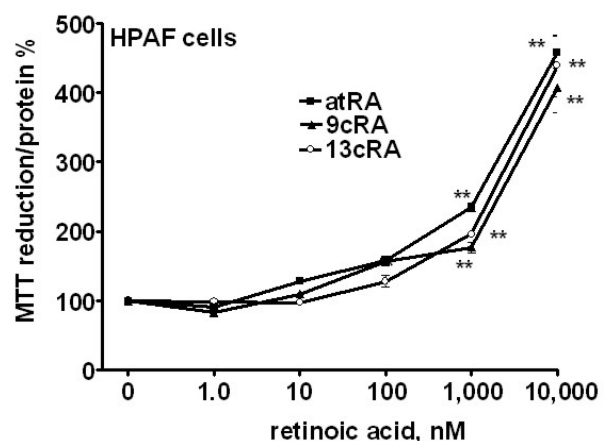

Figure 2. Induction of the MTT-reducing activity by retinoic acid. The extensive increase in the MTT optical density normalized to total protein content after two days of treatment with three natural isomers of retinoic acid (all-trans-, 9-cis- and 13-cis-) was measured and expressed as percent of untreated controls in pancreatic HPAF cells [4, 10].

It has been suggested that the reduction in mitochondrial differentiation rather than hypoxia modulates the prognosis of cancer cells [9, 12, 15]. Moreover, it has been shown that solid tumors have substantial differences in their metastatic potential although they all are hypoxic [1, 8]. In all tumor types investigated, high molar concentrations of lactate were correlated with a high incidence of distant metastasis already in an early stage of the disease. This was due to the activation of hyaluronan synthesis by tumor-associated fibroblasts, upregulation of the vascular endothelial growth factor and hypoxia-induced factor-1alpha, and the direct enhancement of cellular motility which generates favorable conditions for metastatic spread [9]. Therefore, abnormalities in the cytoskeleton and associated proteins could be the major modifier of mitochondrial biogenesis and activity. Retinoic acid-induced apoptosis in T24 bladder cancer cells was associated with a redistribution of Bax and Bcl-2 proteins in the subcellular compartment. This coincided with the reorganization of the cytoplasmic intermediate filament network. The cleavage of cytokeratins by caspases in this model was associated with aggregation of the mitochondria and lysosome [21]. Moreover, chemical depolymerization of microtubules invariably leads to the inhibition of mitochondrial volume and mass increases during the inter-phase of the cell cycle. The presence of stable microtubules is the most pronounced in various cellular processes which require a large amount of energy: neurite outgrowth; myotube differentiation; formation of a monolayer of epithelial cells; and pressure overloaded cardiac hypertrophy. Stabilization of microtubules by prolonged exposure of the human osteosarcoma cell line 143B cells and rat liver-derived RL-34 cells to taxol induced an abnormal accumulation of mitochondria in cells arrested in the G2/M phase of the cell cycle. Mitochondrial proliferation and degradation have been suggested to depend upon functional states of the organelles or energetic states of the cell. Accordingly, disorganization of these processes is often associated with an abnormal accumulation of mitochondria in various models of cell death. On the contrary, depolymerization of microtubules by nocodazole and colchicine inhibited mitochondrial proliferation during G1 to G2 phase progression and arrested cells in the G2/M phase. Co-treatment of cells with taxol and nocodazole or taxol and colchicines suppressed taxol-induced proliferation of mitochondria as was confirmed by an increase in subunit VIII of human cytochrome c oxidase and by enhanced mtDNA replication. Two subpopulations of mitochondria were detected in taxol-treated cells: mitochondria with high $\Delta\Psi_m$ and those with low $\Delta\Psi_m$, reflecting apoptotic death. Treatment with herbimycin A or H_2O_2, known to induce the accumulation of mitochondria in mammalian cells, causes stabilization of microtubules in 143B cells. Furthermore, the elevation of the mitochondrial mass has been reported in HEP-2 cells cultured in the presence of cytotoxic necrotizing factor 1 (CNNF1), an activator of Rho GTPase, a sufficient factor required for the stabilization of microtubules. Structurally unrelated to nocodazole or colchicines, 2-methoxyestradiol depolymerizes microtubules and at the same time inhibits the proliferation of mitochondria [22]. Leflunomide, an inhibitor of mitochondrial enzyme dihydroorotate dehydrogenase, causes an unrestrained proliferation of mitochondria both in human osteosarcoma cell line 143B cells and rat liver derived RL-34 cells with an increased intracellular level of acetylated alpha-tubulin. In consequence, changes in the physicochemical properties of microtubules may somehow modulate the biogenesis of mitochondria [23].

We observed that antiproliferation and cell-cycle arrest were integral parts of a redifferentiation-apoptosis sequence since low non-redifferentiation concentrations of retinoic acid failed to provoke apoptosis [4]. The proposed aerobic redifferentiation was also confirmed by a few-fold induction of mitochondrial mass compared to controls within 24 hours. The $\Delta\Psi_m$ was normal for at

least three days post treatment. A significant but gradual loss of $\Delta\Psi_m$ was observed thereafter (Figure 3).

Previous reports have shown that cell-cycle arrest and apoptotic cascade induced by butyrate is dependent upon the presence of a normal mitochondrial membrane potential [24]. Also, $\Delta\Psi_m$ elevation, stabilization, or collapse results in delayed, decreased, or blocked apoptosis, respectively [25]. The observed induction of mitochondrial activity and mass suggests an effector role for the mitochondria in the present retinoic acid-induced re-/trans-differentiation and subsequent apoptosis model. The mitochondrial redifferentiation process is a part of a more generalized reprogramming of gene expression toward normal cell behavior. Despite the presence of apoptotic concentrations of retinoic acid, some of these normalized cells were able to functionally transdifferentiate into pancreatic endocrine cells [6]. This reflected the viability of the treated cells and suggested their stem-cell potential to escape apoptosis by transdifferentiation into another cell type. Consequently, mitochondrial redifferentiation may provide energy and/or effector molecules for the whole process, including the repair of DNA abnormalities. Apoptosis was induced in as a final fate because of the inability of the cells to repair the damaged DNA. Similarly, it was reported that retinoids, as a single agent, induce a terminal differentiation phenotype well before cell death by apoptosis in a temporally defined order [26]. The acyclic retinoid, all trans-3,7,11,15-tetramethyl-2,4,6, 10,14-hexadecapentaenoic acid, prevented hepatocarcinogenesis in animal models and in a randomized clinical trial by eradicating premalignant and latent malignant clones of transformed cells from the liver. Production of albumin, a redifferentiation marker, was recovered while that of lectin-reactive isoform of alpha-fetoprotein, a dedifferentiation marker, was reduced after treatment of human hepatoma-derived cell lines for two days [27]. Normal spatial and temporal patterns of cell proliferation, differentiation, and apoptosis in the colonic mucosa are determined by
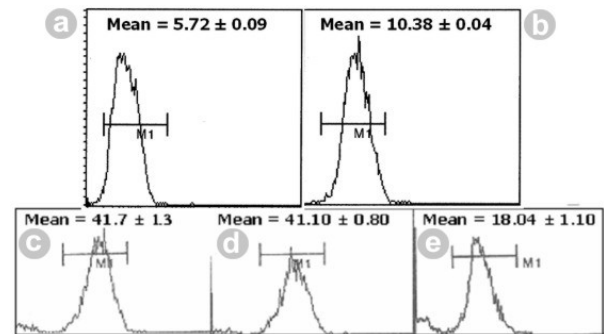

Figure 3. Increased mitochondrial mass and changes in mitochondrial membrane potential induced by all-trans-retinoic acid. Mitochondrial mass was doubled in HPAF cells treated with the highest apoptotic concentration used, 10 μM for 24 h (**b.**), compared to the untreated control (**a.**) [4]. Mitochondrial membrane potential was normal after 24 h of treatment (**d.**), but was gradually reduced to reach significance at 3 days (**e.**) compared to the control (**c.**) [5].

developmentally programmed genetic and external signals generated by homo- and heterotypic cell interactions, humoral agents, and luminal contents. Mitochondrial function may play a pivotal role in integrating these signals and in determining the probability of cells entering different maturation pathways [28]. Both doxorubicin and mitoxantrone were differentially potent inducers of apoptosis in H9C2 cardiomyocytes and MTLn3 breast cancer cells. In particular, the two drugs induced a progressive increase in mitochondrial mass in the cancer cells but not in the cardiac cells. The mitochondrial proliferation preceded the nuclear apoptosis. The proliferation of mitochondria could explain the higher toxicity of doxorubicin to cancer cells compared to cardiac cells [29]. Elevated mitochondrial gene expression is an early event in the switch from proliferation to differentiation of the human colon adenocarcinoma cell line, HT29, promoted by trehalose replacement of exogenous glucose [30, 31]. The expression levels of COXIII, a mitochondrial gene encoding one of the 13 subunits of cytochrome c oxidase, are abnormally low in colonic tumors and colonic tissue at genetic risk for developing tumors. It was increased in HT29 human colonic adenocarcinoma cells treated with butyrate that may enhance the potential for cellular respiration [32]. Herbimycin-treated breast
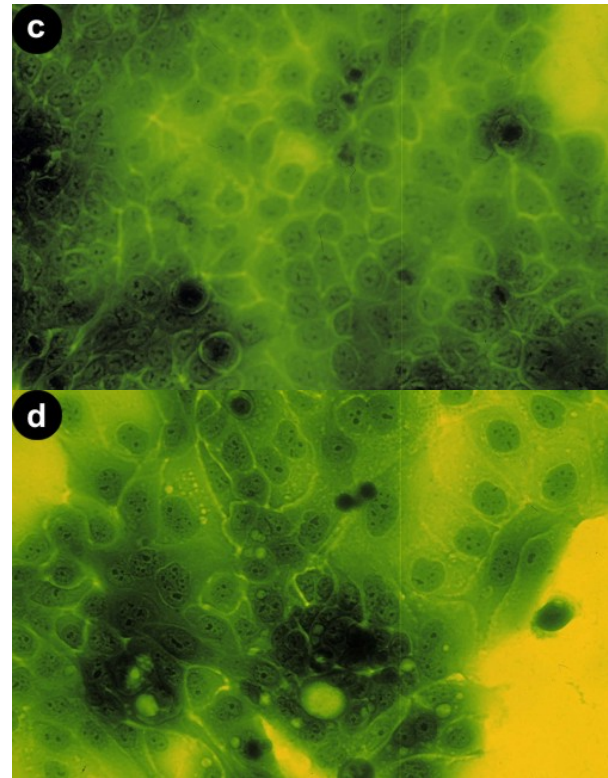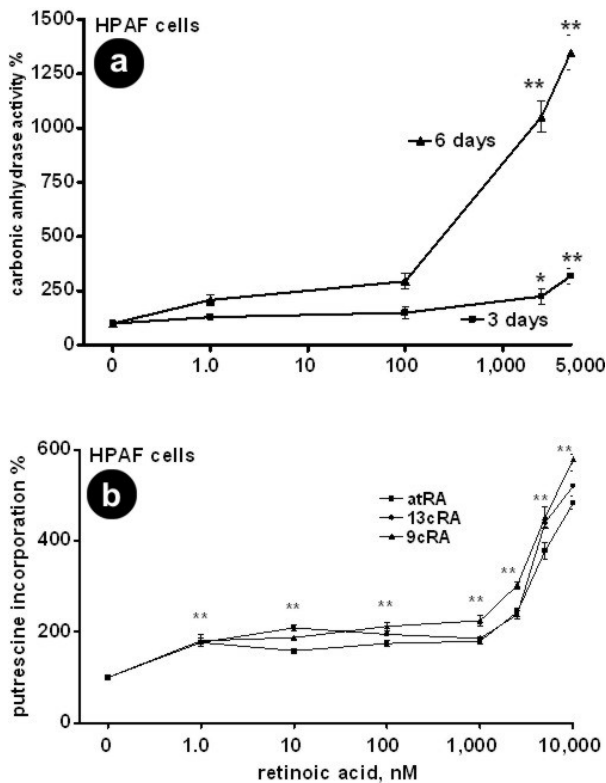
Figure 4. Induction of terminal biochemical and phenotypic differentiation by retinoic acid. Especially the treatment with the apoptotic concentrations of all-trans-retinoic acid induced extensive carbonic anhydrase II (**a.**) [4], and transglutaminase (for four days, measured as ^{3}H-putrescine incorporation, [5]) (**b.**) activities. The lower panels show the morphology of the control HPAF cells with polygonal unpolarized cells with massive nucleus-cytosol ratio and mitotic figures (**c.**), compared with 2.5 μM all-trans-retinoic acid treated cells for three days with typical polarized simple columnar epithelium with highly reduced nucleus-cytoplasm ration, secretory activity and a few apoptotic bodies (**d.**) [4, 5].

cancer cell line SKBr3 cells showed increased mitochondrial mass, with no corresponding increase in $\Delta\Psi_m$ before undergoing apoptosis [33]. Mitochondrial mass and mitochondrial DNA content were increased with differentiation of human embryonic stem cells, which was accompanied by the increase of the amount of ATP (4-fold) and its by-product reactive oxygen species (2.5-fold) supporting anaerobic-to-aerobic metabolic transition during differentiation [34]. It was demonstrated that the normal mitochondrial respiratory chain (MRC) plays an essential role in the interferon-beta/retinoic acid-induced cancer cell death. These agents up regulate the expression of MRC complex I subunits [35]. In the most successful application of redifferentiation as an anticancer therapy, all-*trans*-retinoic acid induces differentiation of acute promyelocytic leukemias carrying the t(1;19) translocation. Cells that have differentiated remained viable

for a few days and eventually apoptosed. Restoration of a normal differentiation program in cancer cells, in consequence, appears to also activate an apoptotic mechanism similar to the normal physiological process [3].

In our model, few cells treated with the concentration of retinoic acid-causing apoptosis underwent apoptosis at the beginning of the treatment. If these apoptotic cells would have been collected separately and investigated, the expected full-scale terminal differentiation would not have been detected. This emphasizes the value of utilizing earlier mitochondrial redifferentiation to indicate its necessity for apoptosis [36]. Subsequently, the remaining majority of the treated cells exhibited phenotypic and biochemical features of terminal redifferentiation, including increased carbonic anhydrase II and transglutaminase activities (Figure 4) [5]. Similar anaerobic to aerobic reversion has

also been reported during the induction of glucocorticoid-induced apoptosis with a biphasic course of lactate production. Prior to the onset of apoptosis, i.e., prior to the loss of $\Delta\Psi_m$, lactate production was reduced. A massive increase in lactate production was observed in the human acute lymphoblastic leukemia cell line CCRF-CEM, subsequent to the loss of $\Delta\Psi_m$ [37]. Herbimycin A induced redifferentiation of the poorly differentiated colorectal Colo-205 carcinoma cells. Redifferentiation involved unrestrained mitochondrial proliferation and progressive $\Delta\Psi_m$ dysfunction preceding apoptosis [38]. Mitochondrial activity and glucose consumption were significantly stimulated after sodium butyrate-induced peritoneal carcinomatosis cells differentiation and prior to their apoptosis [39]. Ultrastructural examination of the doxorubicin-treated human MCF-7 breast adenocarcinoma cells revealed morphological alterations consistent with the induction of differentiation (e.g., increased lipid content and mitochondrial density, appearance of tight junctions, and secretory ducts) with a subsequent gradual loss of cells through apoptosis [40]. The differentiation of the inducible murine neuroblastoma C1300 clone, N1E-115, was associated with an important increase of the cellular content in mitochondria. This increase could be observed with differentiating N1E-115 cells maintained in suspension, i.e., under conditions where neurite outgrowth is prevented but other early stages of (biochemical) differentiation continue to occur [41]. Uncoupling redifferentiation and apoptosis in our model by using TGF-beta neutralizing antibodies, using retinoid antagonists and utilizing low non-redifferentiating concentration of retinoic acid, all inhibited apoptosis. Therefore, confirming the suggested obligatory Transformation - Normalizing-redifferent-iation - Apoptosis sequence at least in the present model [4, 5, 6, 10].

Nevertheless, several studies on cytotoxic treatment inducing apoptosis found a loss of mitochondrial membrane potential with or without cytochrome c release, oxidative stress, activation of caspases, increase in annexin V binding and DNA fragmentation without prior tissue-specific differentiation. This sequence of events could be true when the timeframe, redifferentiation indicator(s), cell type and apoptotic inducer are considered. In these models, the induction of confirmed apoptosis might have been preceded by redifferentiation, if earlier redifferentiation markers at earlier time points and lower concentrations of the apoptotic inducer would have been used. For example, cytokinins (plant redifferentiation-inducing hormones) were very effective in inducing mature granulocytes in the human myeloid leukemia HL-60 cells. On the other hand, cytokinin ribosides were the most potent substances for growth inhibition and apoptosis by greatly reducing the intracellular ATP content and disturbing the $\Delta\Psi_m$ and the accumulation of reactive oxygen species, not observed with cytokinins. Coincubation with the O_2^- scavenging antioxidant or caspase inhibitor significantly reduced apoptosis. Reduction and delay of induction of apoptosis disclosed the masked degree of differentiation with the ribosides along full-scale granulocytic phenotype [42]. Therefore, in such a model, the meaning of redifferentiation would depend largely on the stage at which the respective biomarker would be expressed and detected.

If biologically basic and general biomarkers, such as transient increases in oxidative mitochondrial activity or its effector subunits, mitochondrial mass and normal potential were investigated at a tight timeframe, it could be found that redifferentiation preceded apoptosis. After differentiation of murine erythroleukemia there was an initial over expression of mitochondrial oxidative phosphorylation complexes II and IV mRNAs, followed by a gradual decline after 36 hours of incubation with dimethyl sulfoxide (DMSO) and/or 2-(3-ethylureido)-6-methylpyridine [43]. This is normal because apoptosis is the physiological fate of a normal differentiated cell or a normalized redifferentiated cell. Joshi *et al.* [44] reported that a functioning mitochondrial respiratory chain was required for cellular sensitivity to BMD188, a novel

prostate cancer chemotherapeutic drug. Resveratrol, a plant polyphenol, triggers a p53-independent apoptotic pathway in the colon carcinoma HCT116 cell line which may be linked to differentiation, since apoptosis was preceded by mitochondrial proliferation and signs of epithelial differentiation. Physiological concentrations of n-butyrate induced apoptosis independently of p53 in the HCT116 colon carcinoma cell line. The apoptosis was mediated through the mitochondria and was accompanied by mitochondrial proliferation and $\Delta\Psi_m$ changes [45]. Reipert *et al.* [46] also reported mitochondrial proliferation and increased mass at all stages of the cell cycle in the etoposide treated FDCP-mix, a pluripotent murine hematopoietic stem cell line. Subsequently, there was a decline in mitochondria mass and $\Delta\Psi_m$ in the later stages of apoptosis with G2 arrest. The G2/M phase arrest was associated with an increase in $\Delta\Psi_m$, whereas treatment with a ten-fold higher drug concentration triggered massive apoptosis and a collapse of $\Delta\Psi_m$ [47]. At the late stage of etoposide-induced apoptosis in HL-60 cells, mitochondria increased in numbers as an integral part of a cascade of apoptotic events. There was also a drastic increase in mitochondrial DNA content with decreased $\Delta\Psi_m$ and ATP content. The increase in mitochondrial DNA levels correlated with an elevated expression of one of the regulators of mitochondrial DNA replication, mtSSB [48]. Treatment of U937 cells with the flavonoid quercetin elicited three cell populations with different $\Delta\Psi_m$: 1) healthy cells, with normal $\Delta\Psi_m$, DNA content and physical parameters, and high mitochondrial mass without apoptosis; 2) cells with intermediate $\Delta\Psi_m$ and normal DNA content, but with physical parameters typical of apoptotic cells and low mitochondrial mass; and 3) cells with collapsed $\Delta\Psi_m$ that had low mitochondrial mass and were apoptotic. They represent different stages of preapoptosis and apoptosis, respectively [49].

An exception of the proposed Transformation - Normalizing-redifferentiation - Apoptosis sequence could be expected during the induction of apoptosis in the hemoblastoses characterized by a specific stage of the so-called "frozen differentiation" rather than dedifferentiation as seen in solid tumor cells [50]. Therefore, their apoptosis may be induced without further *de novo* differentiation, utilizing the basic degree of differentiation already present. However, even in such model there is redifferentiation at the mitochondrial and tissue-specific metabolic and morphological levels. Accordingly, continuous exposure to the antimetabolite 1-beta-D-arabino-furanosylcytosine inhibited proliferation and induced expression of the myelomonocytic differentiation marker CD11b in approximately 35% of human myelomonocytic leukemia U937 cells [51]. Arsenic trioxide exerted dose-dependent dual effects on acute promyelocytic leukemia cells. Rapid apoptosis was evident when cells were treated with 0.5-2.0 μM of arsenic trioxide, while partial differentiation was observed using low concentrations (0.1-0.5 μM) of the drug [52]. The histone deacetylase inhibitor, sodium butyrate, induced cell-cycle arrest and differentiation followed by apoptosis in U937 human monocytic leukemia cells [53]. In dexamethasone-induced apoptosis of the human acute lymphoblastic leukemia cell line CCRF-CEM, at least 24 h prior to and up to the point of apoptosis detection (36 h), concomitant with redifferentiation and prior to the loss of $\Delta\Psi_m$, lactate production proportional to viable cell number was reduced compared to untreated controls. However, a massive increase in lactate production was observed, subsequent to a loss of $\Delta\Psi_m$. A similar pattern was observed in other cell lines (HL60, THP1 and OPM2) with various cytotoxic agents (doxorubicin, gemcitabine and vumon (VM26)) [37]. K562 erythroleukemia cells undergo apoptosis when induced to differentiate along the erythroid lineage with hemin [54]. Reflecting mitochondrial redifferentiation, lactate accumulation in the medium and glucose utilization decreased during the induction of *in vitro* differentiation of mouse erythroleukemia and human myeloid leukemia HL-60 cells [55].

In conclusion, the effect of the retinoid on pancreatic adenocarcinoma cells as a model for a Transformation - Normalizing-redifferentiation - Apoptosis sequence suggests the important role of mitochondria and confirms the results from several redifferentiation-apoptosis models that utilized different types of cells and apoptotic inducers. The necessity of such a sequence mandates further molecular investigations.

Keywords Apoptosis; Cell Differentiation; Mitochondria; MTT formazan; Retinoids

Abbreviations MRC: mitochondrial respiratory chain; MTT: 3-(4,5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide

Correspondence
Tarek Hassan El-Metwally
Department of Medical Biochemistry
Faculty of Medicine, Assiut University
Assiut
Egypt
Phone: +20-88.230.9566
Fax: +20-88.233.2278
E-mail: thelmetwally@hotmail.com

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