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Review

Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells

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ABSTRACT

Apoptosis is a highly regulated mechanism by which cells undergo cell death in an active way. As one of the most challenging tasks concerning cancer is to induce apoptosis in malignant cells, researchers increasingly focus on natural products to modulate apoptotic signaling pathways. Curcumin, a natural compound isolated from the plant *Curcuma longa*, has chemopreventive properties, which are mainly due to its ability to arrest cell cycle and to induce apoptosis.

This article reviews the main effects of curcumin on the different apoptotic signaling pathways involved in curcumin-induced apoptosis of cancer cells, including the intrinsic and extrinsic apoptosis pathways, the NF-κB-mediated pathway as well as the PI3K/Akt signaling pathway. This review also focuses on the sensitization of cells to TRAIL-induced apoptosis after curcumin treatment and shows that curcumin enhances the capacity to induce cell death of different chemotherapeutical drugs.

Contents

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1. Introduction

Apoptosis is an ubiquitous and highly regulated mechanism by which cells undergo programmed cell death [1]. Resistance to apoptosis is a hallmark of cancer, with both the loss of pro-apoptotic signals and the gain of anti-apoptotic mechanisms contributing to tumorigenesis [2]. Several cellular pathways cumulate in the activation of caspases and apoptosis. Overly simplified, the two main apoptosis pathways are the extrinsic and the intrinsic pathway [3]. The extrinsic pathway is initiated by the interaction between specific ligands and surface receptors [4], such as CD95/Fas/Apo1, tumor necrosis factor (TNF) receptor 1 (TNFR1), TNF receptor 2 (TNFR2) and death receptors 3–6 (DR3–6) [1], which are able to deliver a death signal from the extracellular microenvironment to the cytoplasm. Binding to the receptor induces receptor multimerization, binding of Fas-associated death domain (FADD) adapter protein, formation of the death-induced signaling complex (DISC) which recruits the initiator caspases 8 and 10 and subsequently activation of the effector caspases 3 and 7 [4].

The intrinsic pathway is activated by various stimuli, i.e. DNA damage, hypoxia, cell detachment, cellular distress and cytotoxic drugs, which act inside the cell [1]. All of these signals converge to mitochondria, where the propagation of the apoptotic signal is regulated by the Bcl-2 family members [5]. Bcl-2 and Bcl-xL exert anti-apoptotic effects, while others such as Bid, Bad and Bim are pro-apoptotic [6,7]. An excess of pro-apoptotic over anti-apoptotic signals initiates mitochondrial outer membrane permeabilization (MOMP), which leads to the release of proteins such as cytochrome *c* and Smac/Diablo from the mitochondrial intermembrane space to the cytosol. Once cytochrome *c* is released, it binds to Apaf-1 and ATP, which then bind to pro-caspase 9 to create a protein complex known as apoptosome, which in turn activates the effector caspase 3 [8]. Smac binds to the inhibitor of apoptosis proteins (IAPs) and deactivates them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed.

A third apoptotic pathway, the ''endoplasmic reticulum (ER) stress'' pathway has recently been described [9]. ER stress, induced by the accumulation of unfolded or malfolded proteins due to hypoxia, nutrient deprivation, reduction of disulfide bonds, and over-expression of some proteins, activates various apoptotic pathways. Crosstalk between the mitochondria and ER plays an essential role in ER stress-mediated cell death. The cytochrome *c*-dependent apoptotic pathway is activated by ER stress [10]. On the other hand, caspase-12, which is located at the ER, is also activated by excess ER stress and results in cell death in the absence of the cytochrome *c*-dependent pathway [10].

The existence of various check-points in apoptosis reveals a complex balance between cell survival and cell death in cells.

Two of the main signaling pathways involved in cell survival, by inhibiting apoptotic processes, are the nuclear factor-kappa B (NF-κB) and the Akt signaling pathways. The transcription factor NF-κB is one of the most studied transcription factors in mammalian cells. Its function has been implicated in inflammation, cell proliferation, differentiation, apoptosis and cell survival. NF-κB is an ubiquitously expressed family of five proteins; p65 (RelA), p50, p52, c-Rel and RelB.

Many stimuli give survival responses to cells that are mediated by NF-κB. Indeed, overall reduction in NF-κB activity is associated with increased apoptotic index in many cell types [11]. Furthermore, NF-κB activation has been shown to inhibit p53 dependent apoptosis following expression of the oncogene AP12/MALT1 [12]. This NF-κB directed survival response is associated with increased expression of anti-apoptotic proteins. Thus, it is not surprising that NF-κB expression is deregulated in various disease states including chronic inflammation and cancer.

The phosphatidylinositol-3-kinase (PI3K) signaling pathway is also crucial for many aspects of cell growth and survival and is frequently disrupted in human cancers. The PI3Ks are a family of related enzymes that are capable of phosphorylating the 3 position hydroxyl group of the inositol ring of PI(4,5)P2, to generate PI(3,4,5)P3 [13]. Upon activation of the PI3K pathway by many growth factors (e.g. epidermal growth factor (EGF)) and regulators, PI(3,4,5)P3 is produced on the inner side of the plasma membrane and Akt binds to the phospholipids. Akt, also called PKB (Protein kinase B) or RAC, (related to protein kinase A and C), is the human homologue of the viral oncogene *v-akt* ([14–16] which regulates multiple targets including several apoptotic genes [17,18]. Akt inactivates pro-apoptotic factors like Bad, which controls the release of cytochrome *c* [19,20], procaspase-9 and Forkhead transcription factors (like FOXO). Akt also activates anti-apoptotic genes, including cyclic-AMP response element-binding protein (CREB) and IκB kinase (IKK) leading to NF-κB nuclear localization and the subsequent transcription of pro-survival genes, such as Bcl-xL, caspase inhibitors and c-Myb [21,22]. Overexpression of Akt has anti-apoptotic effects in various cell types resulting in cell death resistance [23].

Curcumin, a phenolic compound isolated from the plant *Curcuma longa* has anti-inflammatory, antioxidant and anti-cancer activities. The anticarcinogenic properties of curcumin in animals have been demonstrated by its inhibition of tumor initiation [24] and tumor promotion [25,26]. Although the precise mode of action of this compound is not yet elucidated, studies have shown that chemopreventive action of curcumin might be due to its ability to induce apoptosis by several pathways. The number of signaling pathways and molecular targets involved is continuously growing and consequently the picture is becoming more and more complex, not least

because results often appear to be cell-type specific and dose-dependent.

In order to characterize apoptotic genes regulated by curcumin in tumor cells, Ramachandran et al. have performed a microarray study [27]. Of the 214 apoptosis-associated genes in the array, the expression of 104 genes was altered by curcumin treatment [27]. These results show that curcumin-induced apoptosis is regulated by multiple signaling pathways.

2. Signaling pathways implicated in curcumin-induced apoptosis

2.1. *The intrinsic (mitochondrial) pathway*

The intrinsic (mitochondrial) pathway is initiated in response to cellular signals resulting from DNA damage, a defective cell cycle, detachment from the extracellular matrix, hypoxia, loss of cell survival factors, or other types of severe cell stress. This triggers activation of specific members of the pro-apoptotic Bcl-2 protein family involved in the promotion of apoptosis, Puma and Noxa, which in turn activate the multi-domain pro-apoptotic proteins Bax or Bak. These two proteins move to the mitochondrial membrane and disrupt the function of the anti-apoptotic Bcl-2 proteins thereby allowing permeabilization of the mitochondrial membrane [28].

In the last years, more and more targets of curcumin have been discovered in the intrinsic pathway (Fig. 1).

Indeed, curcumin induces up-regulation of pro-apoptotic proteins of the Bcl-2 family Bax, Bim, Bak, Puma and Noxa and down-regulates, two anti-apoptotic proteins, Bcl-2 and Bcl-xL in cancer cells [29–33]. In U937 cells, curcumin induces apoptosis through the down-regulation of Bcl-xL and IAP proteins, release of cytochrome *c*, activation of caspase-3 and acts as a stimulator of intracellular Ca^{2+} uptake into mitochondria *via* uniporter pathway [29]. Woo et al. have found that curcumin induces apoptosis in human renal Caki cells through the generation of ROS, the down-regulation of Bcl-xL and IAP, the release of cytochrome *c* and the inhibition of Akt [33]. Shankar et al. described a role for Akt in modulating the direct action of p53 on the caspase-dependent mitochondrial death pathway and suggests that these important biological molecules interact at the mitochondrial level to influence curcumin sensitivity in prostate cancer cells [32].

Moreover, curcumin is able to decrease the number of aberrant crypt foci (ACF) in an azoxymethane (AOM)-induced rat colon cancer model through apoptosis *via* the mitochondrial pathway [34]. In HL-60 cells and human colon cancer cells, curcumin induces reactive oxygen species (ROS) and Ca^{2+} productions, decreases the levels of matrix metallo-proteinases (MMP) and Bcl-2, increases the level of caspase 3, Bax and the release of cytochrome *c* leading to apoptosis [35,36].

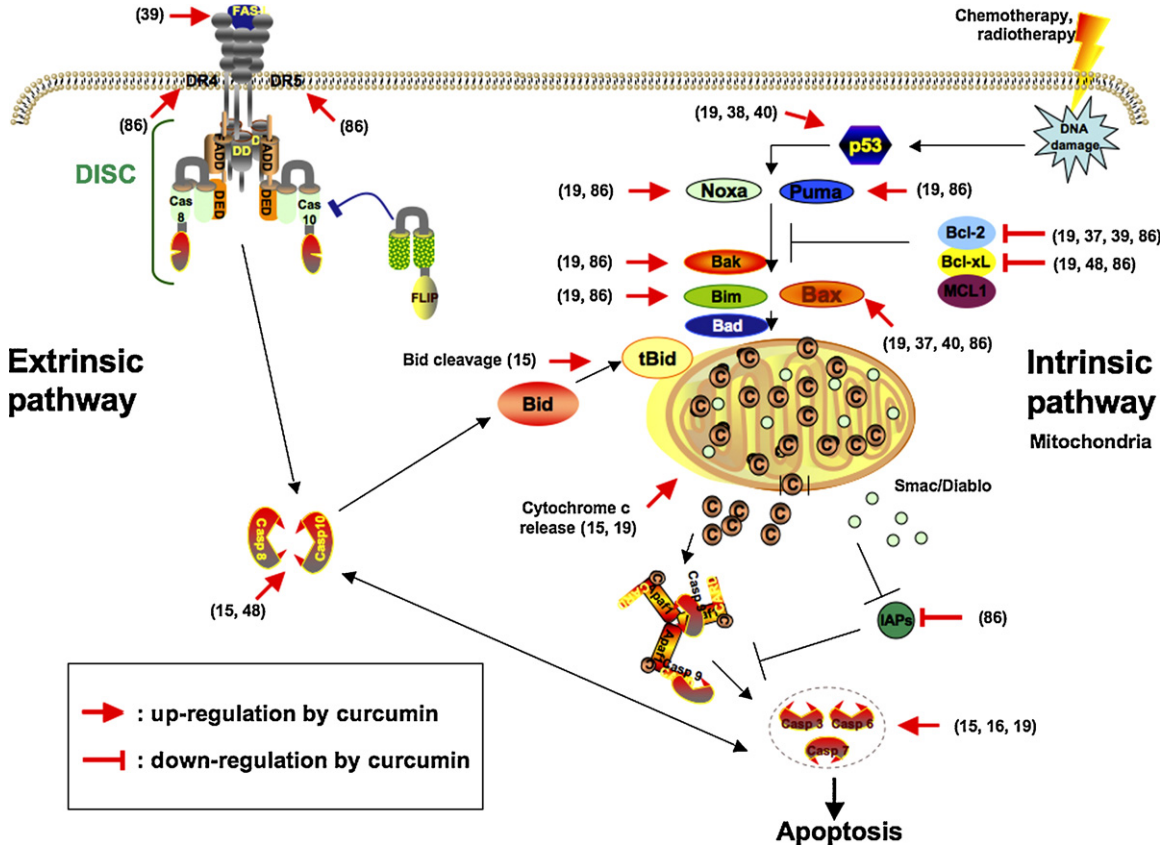

Fig. 1 – Representation of curcumin targets in extrinsic and intrinsic apoptosis pathways. Curcumin regulates proteins of the extrinsic pathway, like Fas, the death receptors (DR) 4 and 5, caspase 8 as well as of the intrinsic mitochondrial pathway including, p53, Puma, Noxa, Bak, Bim, Bad, Bax, Bcl-2, Bcl-xL, IAP and caspase 3. References are indicated. Figure was generated by using ScienceSlides software with modifications.

It has been suggested that curcumin induces apoptosis in tumor cells by increasing the permeability of the mitochondrial membrane. Ligeret et al. confirmed these observations and add that curcumin induces mitochondrial swelling, the collapse of Deltapsi, the release of cytochrome *c* and opened the permeability transition pore (PTP) [37]. Moreover, they demonstrated that curcumin-promoted PTP opening involves the reduction of Fe^{3+} to Fe^{2+}, inducing hydroxyl radical production and oxidation of thiol groups in the membrane [37].

2.1.1. *Implication of reactive oxygen species production*

Curcumin is known for its antioxidant properties and acts as a free radical scavenger by inhibiting lipid peroxidation and oxidative DNA damage. Several *in vitro* studies, however, suggest that curcumin-induced apoptosis is associated with ROS production and/or oxidative stress in transformed cells. Indeed, curcumin was found to induce apoptotic cell death in promyelocytic leukemia HL-60 cells by the generation of ROS through the reduction of copper causing activation of oxygen molecule, which is normally related to the binding ability of curcumin due to the conjugated β-diketone structure [38]. In these cells, curcumin-induced apoptosis by generation of ROS was also shown to be accompanied by a decrease of Bcl-2 [39].

Induction of apoptosis by curcumin observed in human breast epithelial cells involved down-regulation of Bcl-2 and up-regulation of Bax as well as generation of ROS which suggests redox signaling as a mechanism responsible for curcumin-induced apoptosis in these cells [40].

In AK-5 tumor cells, the antitumor activity of curcumin was shown to be linked to the induction of apoptosis by the activation of caspase 3 and through the generation of reactive oxygen intermediates [41,42]. Moreover, due to its physiochemical property curcumin passes easily through the plasma membrane and may cause structural and functional changes in cellular membrane integrity leading to the flipping of phosphatidyl-serine to the outer cell surface which participates in the free radical release [41].

Curcumin-induced apoptosis in MCF-7, MDAMB and HepG2 cells is also mediated through the generation of ROS originating from glutathione depletion by buthionine sulfoximine, thereby further sensitizing the cells to curcumin [43]. In colon cancer cells, curcumin induces apoptosis via a ROS-associated mechanism that converges on JNK activation, and to a lesser extent via a parallel ceramide-associated pathway [44]. Induction of early apoptosis and ROS-generation activity were also observed after curcumin treatment in human gingival fibroblasts and human submandibular gland carcinoma cells [45]. Furthermore, curcumin promoted apoptosis in human skin cancer cells which is preceded by an increase in intracellular ROS production, which supports the notion that mitochondrial respiration and redox tone are pivotal determinants in apoptosis signaling by curcumin in human skin cancer cells [46].

2.1.2. *p53-dependent apoptosis*

More than 50% of human tumors contain a mutation or deletion of the *TP53* gene. The protein encoded by this gene, p53 is a transcription factor that regulates cell cycle and hence functions as a tumor suppressor. It has many anti-cancer mechanisms: it can activate DNA repair proteins when DNA has sustained damage and can initiate apoptosis if the DNA damage was proved to be irreparable. p53 is involved in both the extrinsic and the intrinsic pathways of apoptosis by initiating apoptosis through mitochondrial depolarization and sensitizing cells to apoptosis inducers [47]. The Bcl-2 family has been shown to be a p53 target. Bax, the pro-apoptotic member, is up-regulated in a number of systems during p53-mediated apoptosis [48]. Recent studies have proposed that the alteration of the Bcl-xL/Bax ratio is one of the important factors which decide the fate of a cell [49].

Curcumin was shown to disrupt the conformation of the p53 protein required for its serine phosphorylation, its binding to DNA, its transactivation of p53-responsive genes and p53-mediated cell cycle arrest in colon cancer cells [50].

In human colon adenocarcinoma cells, curcumin up-regulates the serine phosphorylation of p53 and down-regulates the anti-apoptotic factor Bcl-2 and up-regulates the pro-apoptotic factor Bax, thereby decreasing the Bcl-2/Bax ratio and disposing to apoptosis [51]. The same results have been found in breast cancer cells [52]. However, a down-regulation of mutant-type p53 protein and c-myc, Bcl-2, and up-regulation of the expression of Fas by curcumin have been found in human Burkitt's lymphoma [52,53].

In human neuroblastoma curcumin-induced apoptosis is associated with an up-regulation of p53 expression and induced nuclear translocation of p53, followed by the induction of p21(WAF-1/CIP-1), a protein involved in cell cycle, and Bax expression [54].

Moreover, curcumin induced p53-dependent apoptosis in human basal cell carcinoma cells accompanied by up-regulation of its downstream targets p21 CIP1/WAF1 and Gadd45, however, without implication of Bcl-2 and Bax [55]. Because of the mutation in the p53 gene of most tumors, clinically useful antineoplastic agents are less potent and efficacious in the context of mutant p53. In the case of curcumin, Mullally et al., however, showed that curcumin induces cell death independently of p53, indicating that curcumin may be a therapeutical agent for tumors with a p53 mutation [56].

2.2. *The extrinsic (receptor-mediated) pathway*

The extrinsic pathway begins outside the cell through the activation of receptors on the cell surface by specific molecules known as pro-apoptotic ligands, including Apo2L/TRAIL (receptors DR4, DR5), and CD95L/FasL (receptor CD95/Fas) [57,58].

Once activated, the 'death domains' of these receptors, bind to the adaptor protein FADD, resulting in the assembly of the DISC, and recruitment and assembly of initiator caspases 8 and 10 [59] (Fig. 1) .The two caspases are stimulated and processed, releasing active enzyme molecules into the cytosol, where they activate caspases 3, 6, and 7, thereby converging on the intrinsic pathway [60,61].

Bush et al. demonstrated for the first time that curcumin induces apoptosis in human melanoma cells through a Fas receptor/caspase-8 pathway independent of p53 [62]. This was later confirmed by Moragoda et al. as they showed that curcumin induced modulation of cell cycle and apoptosis in gastric and colon cancer cells by initiation of Fas signaling pathway [63]. This leads to the cleavage of PARP and caspase 3,

to the reduction of Bcl-xL levels and the stimulation of caspase 8 activity [63]. In human prostate cancer cell lines curcumin down-regulates cell survival mechanisms (NF-κB, Ap1) and induces apoptosis by down-regulating Bcl-2 and Bcl-XL and activates pro-caspases-3 and -8 [64]. Caspase 8, a key factor of the extrinsic pathway, is also involved in curcumin-induced apoptosis of HL-60 leukemia cells, as well as Bid cleavage, which is the crosstalk between the intrinsic and the extrinsic pathway [65,66].

Activation of extrinsic or intrinsic pathways are not necessarily mutually exclusive but they may be both activated by curcumin in some cell types [67].

2.3. *Curcumin-induced apoptosis through increased stress of endoplasmic reticulum*

Whereas the effect of curcumin on the intrinsic and extrinsic pathways have largely been investigated, recently the involvement of ER stress in curcumin-induced apoptosis has become scientific interest. Some reports demonstrate that curcumin inhibits proteasomal activity [68], triggers the accumulation of cytosolic Ca^{2+} [69,70], and disrupts protein disulfide bond formation [71], all of which trigger an ER stress response. Besides, curcumin induces the expression of ER-associated proteins, like GRP78/Bip and CHOP/GADD153, phosphorylation of PERK and eIF2a in HL-60 and mouse melanoma cells, indicating that curcumin-induced apoptosis may be associated in some cells, at least in part, with its ability to cause ER stress [71,72].

In conclusion, studies performed on apoptosis and curcumin point out that curcumin may induce apoptosis in cancer cells through all the main apoptotic pathways. The predominant apoptotic pathway targeted by curcumin may differ between cell type, differentiation stage or curcumin concentration. The exact mechanism is also difficult to determine because of the crosstalks between the different apoptotic pathways.

2.4. *Inhibition of the survival pathways NF-κB and Akt*

Whereas the previous chapters focused on the effect of curcumin on apoptosis, the following chapter will present studies that investigated the effect of curcumin on survival pathways.

Many studies summarized beneath have shown in the last 10 years that curcumin can inhibit the activity of NF-κB and thereby increase apoptosis.

Indeed, suppression by curcumin of the transcription factor NF-κB, which is constitutively active in CD138+ cells derived from multiple myeloma patients, leads to apoptosis [73]. In melanoma cells, NF-κB and IkappaB kinase (IKK) are constitutively active and curcumin down-regulates these activities [74,75] which is correlated with the anti-proliferative and pro-apoptotic effects of curcumin.

NF-κB activation was inhibited by curcumin through abrogation of IKK and consequently the expression of various cell survival and cell proliferative genes, including Bcl-2, cyclin D1, IL-6, COX-2 and MMP-9, was suppressed [76]. This in turn inhibits proliferation, arrests cell cycle and induces apoptosis of cell carcinoma cells [76]. In human pancreatic cells, NF-κB and IkappaB (IκB) are constitutively active and their down-regulation by curcumin is associated with the suppression of proliferation and the induction of apoptosis [77].

Rhotekin overexpression in human gastric cancer leads to constitutive activation of NF-κB through the phosphorylation of IκB by IKKβ [78]. This overactivated Rho/RTKN/NF-κB signaling pathway may play a key role in gastric tumorigenesis by conferring cell resistance to apoptosis.

Moreover, curcumin can suppress the growth and induces apoptosis of bladder cancer cells *in vitro* through down-regulation of NF-κB and cyclin D1, a protein involved in cell cycle [79]. Overexpression of cyclin D1 and constitutive active NF-κB and IκB genes are the genetic background of human mantle cell lymphoma (MCL) [80]. Curcumin inhibits these activities and induces apoptosis in these cells as well as in human T-cell leukemia virus type I-infected T-cell lines, primary adult T-cell leukemia cells and human malignant astrocytoma cell lines [81,82].

Most chemotherapeutic agents, like paclitaxel, activate NF-κB but curcumin inhibited paclitaxel-activated NF-κB in breast cancer cells as well as the paclitaxel-induced expression of anti-apoptotic, proliferative and metastatic proteins and enhances apoptosis [83]. In a human breast cancer xenograft model, curcumin significantly decreased the incidence of breast cancer metastasis to the lung and suppressed NF-κB, COX-2 and MMP9 [83].

Several groups have demonstrated that curcumin can also inhibit the PI3K/Akt signaling pathway and consequently induces apoptosis of cancer cells. Indeed, Hussain et al. have shown that curcumin induces apoptosis in acute T cell leukemias *via* inhibition of PI3K/Akt pathway [84]. They show that curcumin causes dephosphorylation of constitutively active Akt, FOXO transcription factors, which are a subgroup of the Forkhead family of transcription factors and GSK3, another target of Akt. Gururajan et al. reported that curcumin inhibits cell growth of B lymphoma through inhibition of the survival kinase Akt and its target Bad [85]. They showed however, that Akt is not a direct target of curcumin and claim that the spleen tyrosine kinase (Syk) activity is down-regulated by curcumin accompanied by down-regulation of Akt activation. The mammalian target of rapamycin (mTor), a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription [86,87], is another target of Akt pathway that is inhibited by curcumin in a panel of cell lines [86].

Curcumin, furthermore, inhibits the expression of the two subunits of PI3K, p110 and p85, the phosphorylation of Akt and up-regulates the expression of p53 in prostate cancer cells [32]. This allows to establish a role for Akt in modulating the direct action of p53 on the caspase-dependent mitochondrial death pathway.

Activation of membrane kinases like epidermal growth factor receptor (EGFR) by external growth factors (e.g. EGF) stimulates PI3K/Akt pathways. Curcumin inhibits EGF-stimulated phosphorylation of EGFR in breast cells as well as basal phosphorylation of Akt that may facilitate apoptosis [88].

In conclusion, previous studies suggest that curcumin-induced apoptosis is a result of the induction of pro-

apoptotic proteins and the inhibition of anti-apoptotic proteins as well as inhibition of as survival pathways, like NF-κB and Akt. This predisposition makes curcumin a good anti-cancer drug.

3. Curcumin: a good enhancer of the apoptotic potential of chemotherapeutical drugs

Besides the direct effect of curcumin on different apoptosis signaling pathways, curcumin can also induce or accentuate apoptosis indirectly by sensitizing cells to chemotherapeutical drugs.

3.1. *Cancer cell sensitization to TRAIL-induced apoptosis*

TNF-related apoptosis-inducing ligand (TRAIL) is a ligand molecule that binds to the death receptors DR4 and DR5 and induces apoptosis in several tumors both *in vitro* and *in vivo* in a caspase-8-dependent way. However, some tumors are resistant to TRAIL-induced apoptosis. Prostate cancer cells are generally resistant to the induction of apoptosis by anticancer agents and death ligands, like TRAIL. Therefore sensitization of cancer cells by curcumin to TRAIL-induced apoptosis is of high interest.

Several groups have shown that curcumin is able to sensitize cancer cells to TRAIL-induced apoptosis.

Wahl et al. have pointed out that curcumin enhances TRAIL-induced apoptosis in chemoresistant ovarian cancer cells by activating both the extrinsic and the intrinsic apoptotic pathways [89]. Same results were found in LNCaP prostate cancer cells [90]. Deeb et al. showed that in the prostate cancer cells LNCaP, DU145 and PC3, curcumin is able to block the phosphorylation of IκBα which leads to the inhibition of the constitutively active NF-κB and the subsequent enhancement of the sensitivity of prostate cancer cells to TRAIL [91,92]. Furthermore, they demonstrated that activated (phosphorylated) Akt kinase plays an important role in inhibition of NF-κB and sensitization of LNCaP and PC3 cells to TRAIL by curcumin [93].

Shankar et al. confirmed these results in these cells and add that curcumin inhibited the expression of apoptotic genes (Bcl-2, Bcl-xL, survivin and XIAP) and induced the expression of Bax, Bak, PUMA, Bim, Noxa and death receptors DR4 and DR5 in both cell lines [31]. Besides, they demonstrated that curcumin sensitizes TRAIL-resistant LNCaP xenografts *in vivo* to undergo apoptosis by TRAIL [94].

Gao et al. showed that curcumin sensitizes malignant glioma cells to TRAIL-induced apoptosis by cleaving procaspases-3, -8, -9 and release of cytochrome *c* from mitochondria [95].

These studies suggest that curcumin is able to enhance TRAIL-induced apoptosis of cancer cells either resistant to TRAIL or only slightly susceptible to TRAIL by regulating the expression of death receptors and members of the Bcl-2 family and/or by inactivating NF-κB. As curcumin and TRAIL together can activate both the extrinsic and the intrinsic apoptotic pathways, they may circumvent chemoresistance to conventional chemotherapeutic agents.

3.2. *Synergistic pro-apoptotic effects of curcumin and chemotherapeutical drugs*

Chemotherapy drugs are most effective when given in combination (combination chemotherapy). The rationale for combination chemotherapy is to use drugs that work by different mechanisms of action, thereby decreasing the likelihood that resistant cancer cells will develop. When drugs with different effects are combined, each drug can be used at its optimal dose, without intolerable side effects. In the last 10 years, several studies investigated the effect of curcumin together with other drugs on the potential to enhance apoptosis.

Li et al. have found that curcumin in combination with green tea has increased inhibitory effects against oral carcinogenesis in hamsters at the post-initiation stage and this inhibition may be related to the suppression of cell proliferation, the induction of apoptosis and the inhibition of angiogenesis [96]. Another natural product isolated from plants, Taxol, is a good anticancer agent but its disadvantage is its dose-limiting toxicity. Therefore, a combination of Taxol with curcumin could have potential clinical applications. Indeed, Bava et al. showed that a combination of curcumin with Taxol augments anticancer effects in HeLa cells more efficiently than Taxol alone [97]. They demonstrated that this synergistic effect is in part related to the down-regulation of Taxol-induced NF-κB activation and the phosphorylation of serine/threonine kinase AKT pathways by curcumin.

Sen et al. showed that curcumin enhances Vinorelbine mediated apoptosis in human squamous cell lung carcinoma cells *in vitro* by the mitochondrial pathway and enhances chemotherapeutic efficacy of Vinorelbine [98].

Celecoxib is a highly selective cyclooxygenase (COX)-2 inhibitor, which is an anti-inflammatory agent used in many diseases. However, recent studies have shown that their long-term use may be limited due to cardiovascular toxicity. The use of Celecoxib with curcumin shows increased inhibition of cell growth and induction of apoptosis in osteoarthritis synovial adherent cells [99], in colorectal cancer cells [100] and in pancreatic adenocarcinoma cells [101] through a mechanism that involves inhibition of COX-2 activity. Shpitz et al. show that curcumin was also able to augment the growth inhibitory effect of Celecoxib *in vivo* in a rat model of colorectal cancer [102]. This shows that Celecoxib may be used at lower and safer concentrations and may pave the way for a novel combination treatment for many disorders.

Beta-phenylethyl isothiocyanate (PEITC) is a naturally compound that exhibits significant anti-cancer chemopreventive effects. In addition with curcumin, an additive growth-inhibitory effect and induction of apoptosis were observed in human prostate PC-3 cancer cells and PC-3 prostate tumor xenografts by targeting simultaneously EGFR, Akt and NF-κB signaling pathways [103,104].

Howells et al. showed that oxaliplatin and curcumin displayed marked anti-proliferative capacity in HT29 (p53 mutant adenocarcinoma) and HCT116 (p53wt adenocarcinoma) cells [105]. Apoptosis was induced by both agents and up to 16-fold induction of p53 protein was observed in response to this combination [105].

5-Fluorouracil (5-FU) or 5-FU plus oxaliplatin (FOLFOX) remain the backbone of colorectal cancer chemotherapy, but

with limited success. Therefore Patel et al. investigated whether the combination of curcumin with FOLFOX would be a better strategy for colorectal cancer [106]. Indeed, results showed that curcumin together with FOLFOX produced a significantly greater inhibition of growth and stimulated apoptosis of colon cancer HCT-116 and HT-29 cells compared to each compound used alone [106]. These changes were associated with the attenuation of EGFR and Insulin-like growth factor 1 receptor (IGF-1R) signaling pathways. Decreased activation of EGFR, ErbB-2, ErbB-3 and/or IGF-1R was shown to be also responsible for increased cell growth inhibition and stimulation of apoptosis in response to the combination of curcumin plus EGFR related protein (ERRP), a recently identified pan-erbB inhibitor presenting a good potential to be used as a therapeutic agent for colorectal cancer [107]. On the other hand, Kamat et al. described that curcumin potentiates the apoptotic effects of the chemotherapeutic agents (gemcitabine and paclitaxel) and of cytokines (TNF and TRAIL) by suppressing gemcitabine and TNF-induced NF-κB activation in human bladder cancer cells [108]. In pancreatic cancer cells, however, curcumin was shown to augment gemcitabine cytotoxic effect probably through down-regulation of COX-2 and p-extracellular regulated kinase (ERK) 1/2 levels [109].

4. Anti-apoptotic properties of curcumin

Whereas curcumin mostly induces apoptosis in cells, some studies however show that curcumin is also able to inhibit apoptosis.

Somasundaram et al. demonstrated that curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer through the inhibition of ROS generation and the

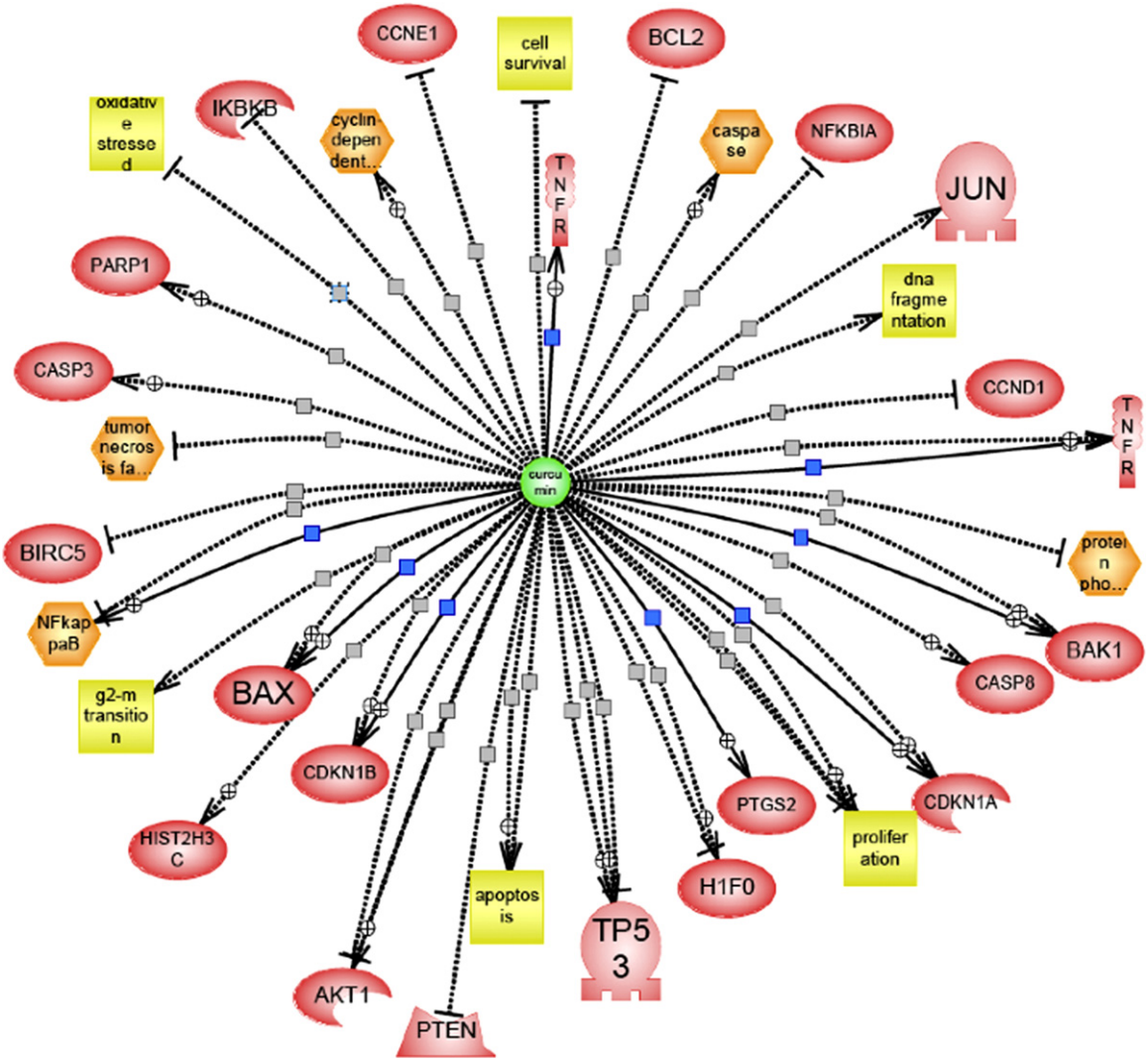

Fig. 2 – Network of proteins, functional protein classes and cell processes involved during curcumin-induced apoptosis. Proteins related to curcumin and apoptosis were extracted from PubMed abstracts using the MedScan Reader software (Version 1.1) [126,127] and then imported to the Pathway Studio software tool (Version 5.0) [128] for visualization. Nodes correspond to proteins (in red), functional classes (in orange) or cell processes (in green). Lines shown correspond to the different types of interactions or relationships between the corresponding nodes and curcumin: expression (marked by a black line with blue central square), regulation (represented by a gray dotted line with gray central square).

blockade of c-Jun N-terminal kinase (JNK) function [110]. They claim that if additional studies would confirm these results, breast cancer patients undergoing chemotherapy should avoid curcumin supplementation.

On the other hand, Chan et al. showed that curcumin inhibits UV irradiation-induced oxidative stress and apoptosis in human epidermoid carcinoma cells which shows in contrast to the previous study a beneficial role for the curcumin-induced decrease of apoptosis [111]. Khajavi et al. showed furthermore that curcumin released the ER-retained myelin protein zero (MPZ) mutants, associated with severe forms of peripheral neuropathy, into the cytoplasm accompanied by a reduced number of apoptotic cells [112,113]. They pointed out that curcumin treatment is sufficient to relieve the toxic effect of mutant aggregation-induced apoptosis and improves the neuropathologic phenotype in an animal model of human neuropathy.

Moreover, Si et al. have found that curcumin-induced disregulation of the ubiquitin-proteasome system (UPS) suppresses replication of coxsackievirus B3 and so protected cells from virus-induced cytopathic effect and apoptosis [114].

Besides, curcumin is able to prevent apoptosis from different apoptosis-inducers, like, copper [115], 1-methyl-4-phenylpyridinium ion (MPP+) [116], IL-1beta [117], methylglyoxal (MG) [118] as well as *tert*-butyl hydroperoxide (t-BHP) [119].

While curcumin may inhibit apoptosis in some cells, in contrast to the pro-apoptotic role described for curcumin, its anti-apoptotic function is generally beneficial for the cell.

5. Curcumin may induce other types of cell death than apoptosis

Although curcumin-induced cell death is mainly due to apoptosis, several other types of mechanisms responsible for curcumin-induced cell death have been described.

Hanif et al. showed that curcumin reduces the proliferation of human colon cancer cell lines without implication of apoptosis [120] and Piwocka et al. described that curcumin induces a novel apoptosis-like pathway independent of mitochondria and caspases in human lymphoblastoid (Jurkat) cells [121]. Whereas the types of cell death induced by curcumin in the previous studies have not been established, curcumin-induced mitotic catastrophe has been described to be responsible for curcumin-induced cell death in HCW-2 cells [122]. Autophagy, designated as programmed cell death type II and characterized by the formation of autophagic vacuoles in the cytoplasm, has been found to be responsible for curcumin-induced cell death in malignant glioma cells [123]. The induced autophagy results from curcumin-induced inhibition of the Akt/mTor/p70S6K pathway and activation of the ERK1/2 pathway [123].

6. Conclusion

Taken together this review summarizes the work performed over the last 12 years on curcumin-induced apoptosis of cancer cells. Much focus has been put on the mechanisms involved during curcumin-induced apoptosis. Several apoptosis signaling pathways and specific proteins have been described playing a role in apoptosis induced by curcumin (Fig. 2) but the precise mode of action of this compound remains to be elucidated. This is due, not only to the dose-dependent effects of curcumin but also to the different mechanisms of action induced as well as to the cell specificity. Besides, crosstalks between the different apoptosis pathways make it difficult to point out the precise apoptosis pathway involved. However, while several targets of curcumin have been described in the different apoptosis pathways, several studies suggest that curcumin mediates its effects through a network of multiple targets in cancer cells that could explain its role in the treatment of various diseases.

In the last years more and more studies demonstrated the increased apoptotic effect of curcumin combined with chemotherapeutic drugs compared to the effect of chemotherapeutic drugs alone. Because it is becoming obvious that the molecular bases for most common diseases are far more complex, this argues against the use of drugs that center on single-target or single-drug approaches. Therefore it is becoming imperative to adopt a multi-target based drug development paradigm for the treatment of complex human diseases [124,125] that work by different mechanisms of action, thereby leading them to decrease the probability that cancer cells will develop resistance against chemotherapeutic drugs.

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