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Curcumin a Multifaceted Compound with Hormetic Behaviour that Mediates an Intricate Crosstalk between Mitochondrial Biogenesis, Mitophagy, Mitophagic Death and Apoptosis

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Abstract

Curcumin, found in the rhizome of turmeric, has extensive therapeutic promises via its antioxidant, anti-inflammatory, and antiproliferative properties. Preclinical *in vitro* and *in vivo* data have shown curcumin to be an effective treatment for multiple cancers. These effects are drived by curcumin's ability to induce G2/M cell cycle arrest, induction of autophagy, activation of apoptotic pathways, disruption of molecular signaling, inhibition of invasion and metastasis, and by increasing the efficacy of existing chemotherapeutics. Here we focused on the hormetic behaviour of curcumin. Frequently, low doses of toxins and other stressors not only are harmless but also activate an adaptive stress whereas high dose activates acute responses like autophagy and cell death. This phenomenon is referred to as hormesis. Many molecules that cause cell death elicite an initial autophagic step that is a cytoprotective mechanism relying on elimination of dysfunctional structures intracellular, notably by mitophagy. This phenomenon is considered as a primarily protective mechanism against stressors. At higher doses, cells undergo mitochondrial outer membrane permeabilization due to calcium release from the endoplasmic reticulum and die. Herein, we address the complex crosstalk between the induced mitochondrial biogenesis, mitochondrial destabilization accompanied by mitophagy and cell death that can also be at play.

Keywords: apoptosis; autophagy; cancer; crosstalk; curcumin; endoplasmic reticulum; hormatic behavior; lysosomes; mitochondria

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Curcumin structural physico-chemistry related to its biological effects

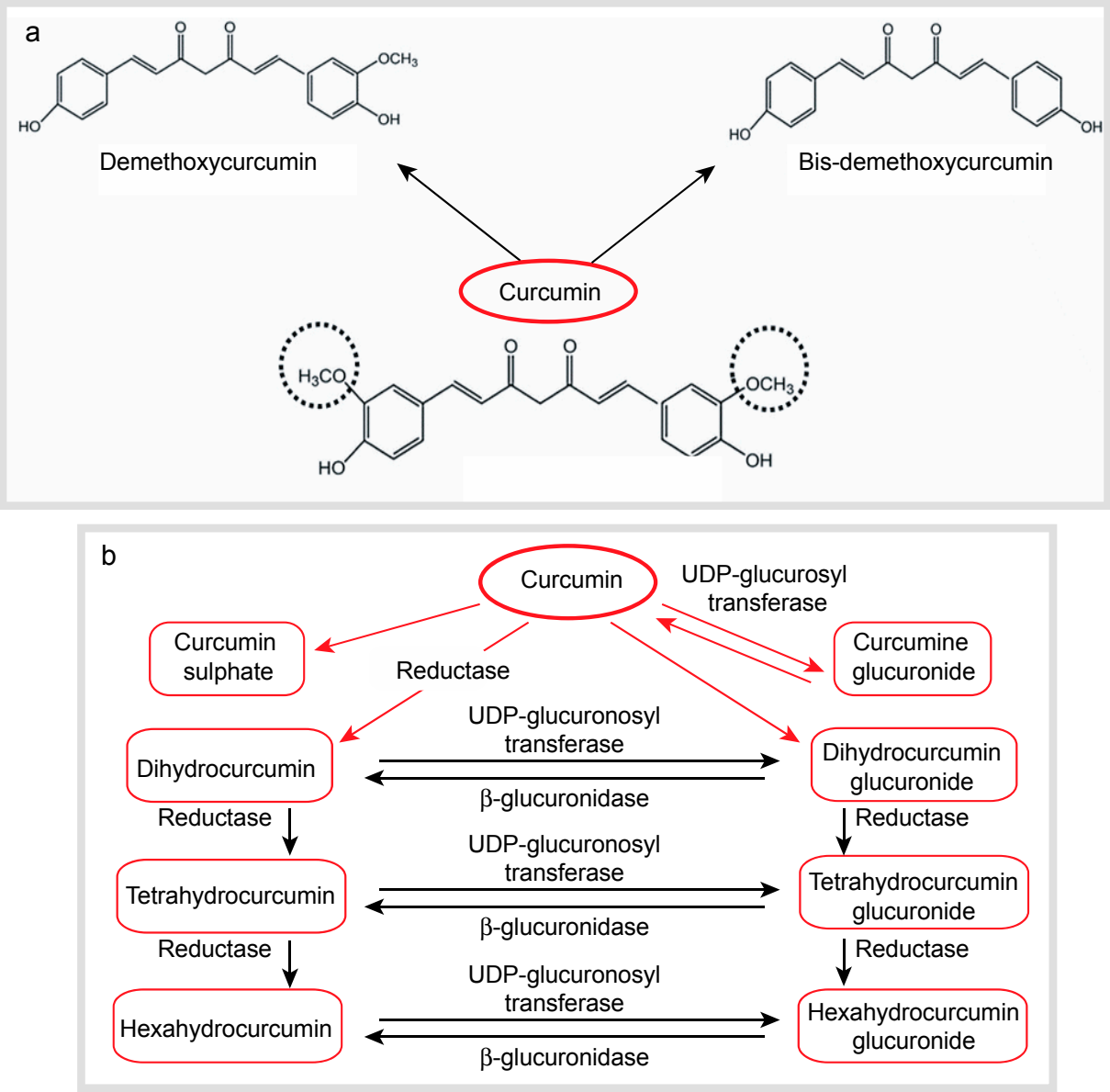

Figure 1

Curcumin is a symmetric molecule, also known as diferuloyl methane [1]. The IUPAC name of curcumin is (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, with chemical formula $C_{21}H_{20}O_6$ (MW 368.38). Its structure contains three chemical entities: two aromatic ring systems with an o-methoxy phenolic groups, connected by a seven carbon linker consisting of an α,β-unsaturated β-diketone moiety (*Figure 1a*). Double bonds inside the molecule resumes it's participation in many electron transferring reactions.

Almost a century after its isolation from turmeric, the first paper on synthesis of curcumin was reported by Lampe in 1913 [2]. The method involved different steps

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starting from carbomethoxyferuloyl chloride and ethyl acetoacetate. Later Pabon [3] described a simple method for the synthesis of curcumin in high yields using acetyl acetone and substituted aromatic aldehydes in the presence of boron trioxide (B_2O_3), trialkyl borate and *n*-butylamine and with slight modifications this method by Pabon [3] has been adopted by several research groups for practically all subsequent curcumin syntheses [4-6] (*Figure 2a*). To improve yields, some patents indicating the use of B_2O_3, trialkylborate and *n*-butylamine along with inert organic amide solvents. Attempts to replace boric oxide with boric acid did not prove to be successful. An alternative method has also been proposed with the use of trifluoroboronite to produce stable curcuminoid trifluoroboronites that can be hydrolysed in aqueous methanol at pH 5.8 to get curcumin [6]. All methods have in common a first step that is the reaction of 2,4-diketones with suitably substituted aromatic aldehydes. To prevent the diketone involvement in Knoevenagel condensation, it is complexed with boron. It is suitable for these reactions to take place in anhydrous conditions and polar solvents, where curcumin can be separated easily from the reaction mixtures. Primary and secondary amines are used as catalysts to provide the necessary basicity to deprotonate the alkyl groups of the diketone. To remove the water produced during the condensation reaction scavengers like alkyl borates are employed. Unless removed, the water can react with the diketone complex, thereby reducing the curcumin yield. In slightly acidic conditions the boron complex formed dissociates into curcumin. Curcumin from this reaction mixture can be separated by repeated washing and precipitation followed by column chromatography. A mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin is obtained (*Figure 1a*). Curcumin can be separated by column chromatography by adsorbing the mixture on silica gel using mixtures of solvents like dichloromethane/acetic acid or methanol/chloroform to yield three different fractions. The curcumin fraction is further purified on silica gel using chloroform / dichloromethane and ethanol / methanol mixtures as eluents [7, 8] Methods for the detection and estimation of curcumin have mostly employed the high performance liquid chromatography (HPLC) technique [8].

Indeed, curcumin is an electron donor and stabilize its chemical structure by redistribution and resonance of the π electron cloud [1]. Moreover and importantly, the extended conjugation confers interesting chemical features to the molecule, including UV-visible absorption bands (250–270 nm and 350–450 nm respectively). So, curcumin fluoresce with emission starting at 470 nm. These optical properties are used for the isolation and purification of curcumin through various techniques, like high performance liquid chromatography (HPLC) and fluorescence enable tracing very low amounts of curcumin and related metabolites in plasma and urine in the range of 2.5 ng/mL [8-10]. The molecule can also be excited at 488 nm with a lower fluorescent yield emission in the 500-530 nm range for detection in flow cytometry and confocal microscopy. Curiously, this has rarely been used for curcumin imaging at the cellular level [11]. The molar extinction coefficient of curcumin is 55000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ at 425 nm in methanol.

Curcumin is weak Brönsted acid, with three labile protons, and accordingly

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three pKa - which can be estimate by both NMR and absorption spectrometry - corresponding to three prototropic equilibria (*Figure 2b*). The first pKa located in the pH range of 7.5 to 8.5 changes curcumin from yellow to red. The chemical reactivity and solubility of the anionic curcumin, *i.e.*, in the basic pH range increase and this form of curcumin is more water soluble than the neutral form.

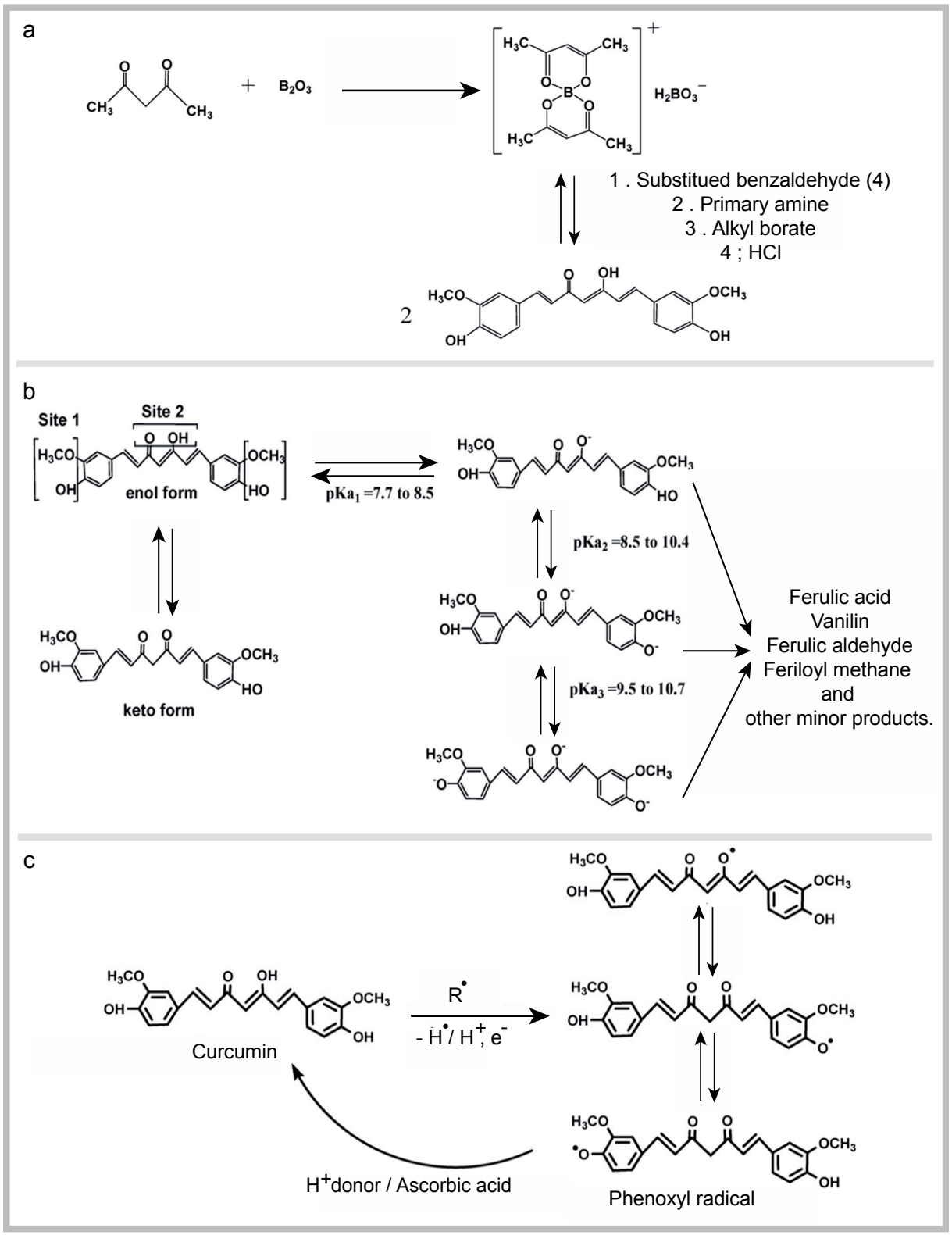

Figure 2

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The key active site of curcumin for biological reactions is the diketo group that acts as the primary hydrogen affinity site at physiological pH [1, 12]. The diketo group displays keto-enol tautomerism to reach prototropic equilibrium [12]. In addition to this enol (A-OH) site, the two phenol-OH locations appear to be more resistant to oxidation, but can undergo oxidation by electron transfer and hydrogen abstraction at more alkaline pH. Curcumin is a hydrophobic molecule with a log P value of 3.0 at neutral pH [13]. Therefore, curcumin is not easily soluble in physiological media and exhibits poor distribution and bioavailability [14].

As a whole, curcumin acts as a hydrophobic reducing agent like vitamin E and scavenge various reactive oxygen species (*Figure 2c*) [14]. Regeneration of phenoxyradicals formed can be achieved by other H^+ donors like ascorbic acid, for consecutive ROS elimination. Curcumin is as efficient in the removal of radicals as well-known antioxidants - Thiols, Vitamin A, Vitamin C and Vitamin E - and mimics the function of superoxide dismutase [13].

The hydrogen donor site, α,β-unsaturated β-diketo moiety, is also considered the breakdown point in the curcumin structure, where curcumin hydrolysis and degradation take place esentially in aqueous physiologic media. Indeed, ninety percent of curcumin degrades within half an hour in water [15], giving rise by hydrolysis to several products, i.e., ferulic acid, ferulic aldehyde, and feruloyl methane.

The rate of curcumin hydrolysis significantly decreases when the diketo reaction site is attached to lipids, peptides, proteins, surfactants, and other macromolecular structures, a situation that often occures within cells [16]. It should be noticed that curcumin solutions are more stable in culture media containing 10% fetal calf serum (FCS). In fact, hydrolytic degradation of curcumin does not effectively occur in the circulatory system since the diketo reactive site is occupied through binding to plasma proteins and other biomolecules [17]. On the other hand, even if the slow catalyzed hydrolytic degradation happens scarcely *in vivo*, it is believed that free cucumin could be subjected to fast enzymatic-facilitated metabolism leading to a hydrophilic metabolites [15]. Curcumin is reduced to tetra-, hexa-, and octa-hydrocurcumins and the two phenolic groups of curcumin are conjugated to produce either curcumin glucuronide or curcumin sulfate [15] (*Figure 1b*). There are proposed pathways for the metabolism of hydrophobic curcumin to hydrophilic metabolites [13].

As a matter of fact, curcumin can ben taken up *in vitro* by THP-1 monocyte / macrophages since its hydrophobicity and readily pass cell membrane[18]. In this context, the subsequent metabolic modification of curcumin to di-, tetra-, hexa-curcumin and its final conjugation to sulfate groups (hexa-curcumin sulfate) is a slow process, providing opportunity for curcumin to exert biological activity and results in lipid accumulation in these cells [18].

Glucuronidation of curcumin and curcuminoids, believed as the phase II metabolism pathway for curcumin in liver, result in the production of varying curcuminoid-glucuronides with diminished biological activity, which do not take place in such cells [19].

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Nonetheless, as the phase I detoxifying enzymes in liver are expressed primarily at higher levels than those of phase II-related enzymes, it was proven that curcumin could induce cellular transcriptional responses associated to enhanced antioxidant capacity of hepatic and extrahepatic tissues. This could explain part the *in*

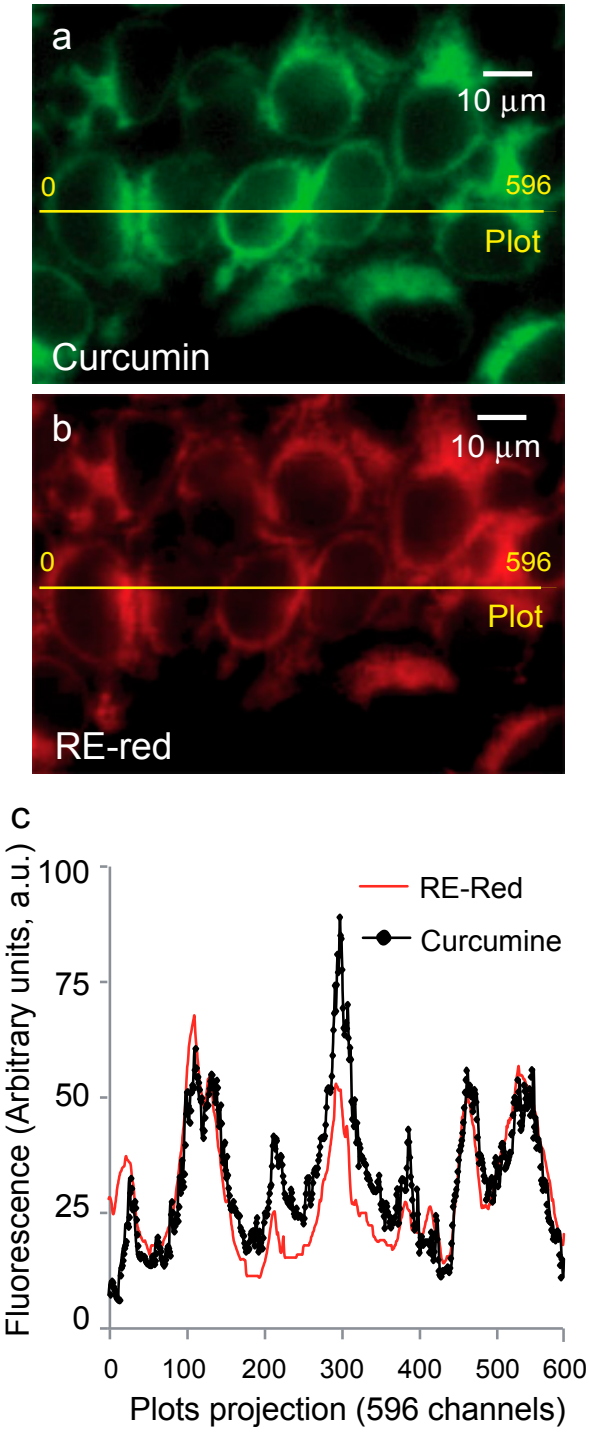

Figure 3

vivo chemopreventive property of curcumin. It should be noticed that despite enhancing antioxidant response of the cells, the other parrallel effects of curcumin (depending of the intracellular concentration of curcumin), i.e., destabilization of the ER membrane and calcium release can ended in mitochondrial destabilization and

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production of more ROS than expected and that these ROS can ovepass the antioxidant defense of the cells.

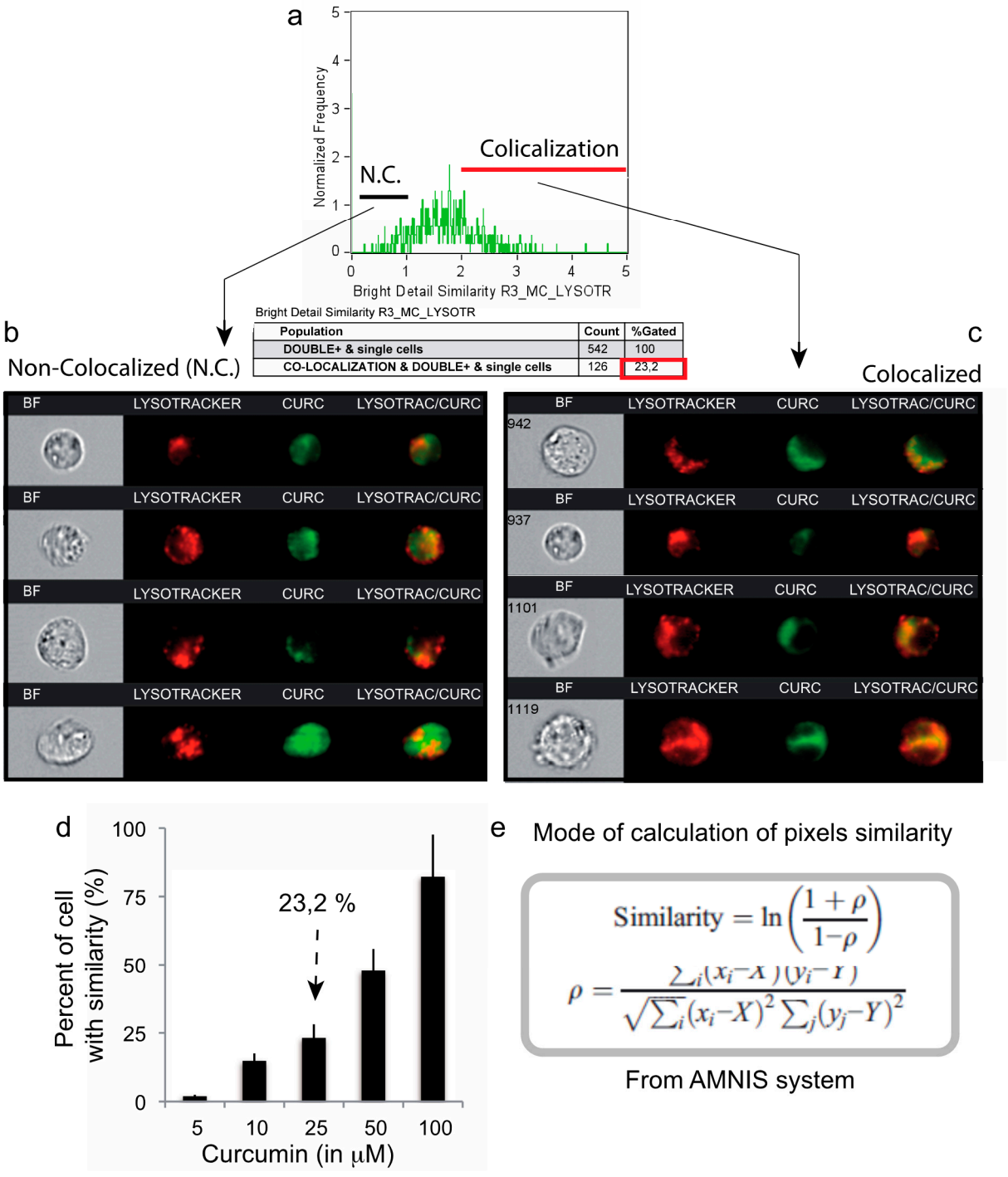

Figure 4

In addition, a nucleophilic addition reaction occurs between the unsaturated ketone of curcumin as an acceptor and anions of A-OH, A-SH, A-SeH from other molecules [13]. As a result curcumin binds covalently to various proteins. For instance, the conjugation of glutathione-SH results in the depletion of the glutathione

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and associated antioxidant defense system in cells. In this regard, the depletion of glutathione molecules suggests curcumin acts as a pro-oxidant contributor in some conditions [15]. Given the significance of the α,β-unsaturated β-diketo moiety, this interesting site of the molecule has been exploited by some chemists for different purposes.

Cellular uptake and intracellular distribution of Curcumin

The cellular distribution of curcumin has not attracted much attention even if curcumin is a fluorescent product and that his localization within the cell or at the level of intracellular membranes might be of great interest to understand its mode of action. Cellular uptake was observed in Huh-7 cells incubated with 20 μM Curcumin after different time intervals ranging from 0 to 48 hours (the experiments have been realized in a medium without pH indicator to avoid any unwanted interferences). To trace the subcellular distribution, curcumin-treated cells were exmined by confocal microscopy or with image cytometry.

The effects of curcumin, including the drop in ΔΨm and the production of ROS, do not appear to be consequences of its direct action on mitochondria [11]. Indeed, the very early release of calcium into the cytoplasm following curcumin treatment prompted us to investigate potential interactions between curcumin and the ER, which contains the main cellular pool of free calcium. Curcumin fluorescence colocalizes perfectly with the ER, which was stained with ER red stain (ER-red) (*Figure 3*). We also compared curcumin fluorescence with the pattern of lysosome staining assessed with LysoTracker Red DN99 (*Figure 4*) some lysosomes colocalizes with curcumin, whereas others did not. Indeed curcumin interactions with lysosomes is strictly dependent of the concentration (*Figure 4*), a situation which explain that the pathway is an additive pathway that may cooperate with the ER, calcium, mitochondrial patway that has been previously described by Moustapha *et al.* [11].

Curcumin-metals complexation reactions

Curcumin forms strong complexes with most of the known metal ions [1]. The α,β-unsaturated β-diketo moiety of curcumin is an excellent chelating agent. In the last one and a half decades, many papers have been published on metal-curcumin complexes : exemples of interactions [18-25] and review papers [1, 20]. Although it is confirmed that curcumin reduces metal toxicity in living systems through complexation, the actual role of these metal complexes in curcumin biology appears to be far more than complex and unclear (*Figure 5*).

More precisely, curcumin can serve as a chelating agent (presumably bidentate) for Fe(II) [26], Fe(III) [27, 28] and Cu(II) [14, 29] (Fig. 5). As a chelating agent of Fe(II), curcumin may prevent reductive cleavage of H_2O_2 to produce hydroxyl radicals ($HO^{\bullet}$) or formation of other reactive oxygen species [26]

A few highlights of chemical structural features associated with the biological activity of curcumin are: - The o-methoxyphenol group and methylenic hydrogen are

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responsible for the antioxidant activity of curcumin, and curcumin donates an electron/hydrogen atom to reactive oxygen species.

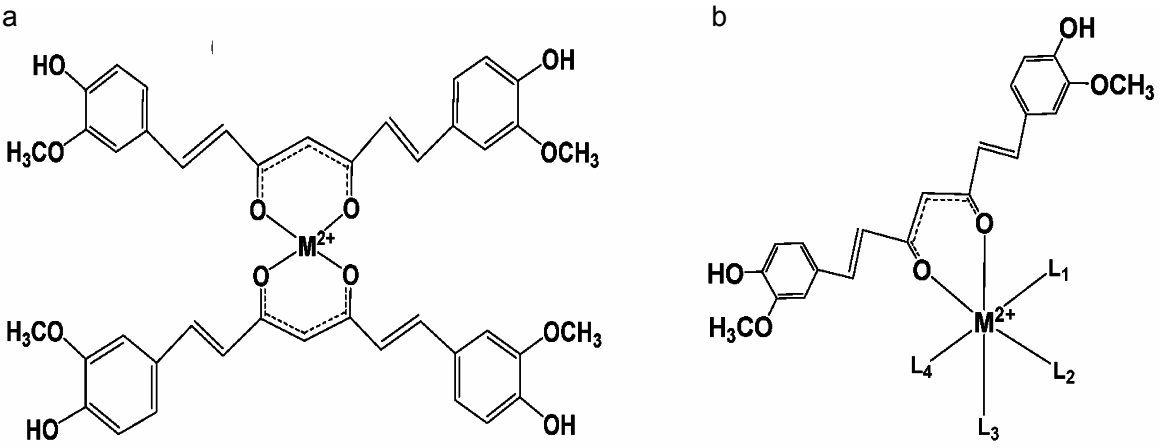

Figure 5

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Curcumin interacts with a number of biomolecules through non-covalent and covalent binding. - The hydrogen bonding and hydrophobicity of curcumin, arising from the aromatic and tautomeric structures along with the flexibility of the linker group are responsible for the non covalent interactions. Precisely, metal complexation with curcumin is mediated by the enolic group with replacement of the enolic proton by the metal in a context where the o-methoxy phenyl group remains unaffected.

Indeed, curcumin forms strong complexes with most of the known metal ions [1]. If curcumin interacts with biomolecules through non-covalent and covalent bindings; the hydrogen bonding and hydrophobicity of curcumin, arising from the aromatic and tautomeric structures along with the flexibility of the linker group are responsible for the non-covalent interactions. The α,β-unsaturated β-diketone moiety covalently interacts with protein thiols, through Michael reaction. But, most importantly, α,β-unsaturated β-diketo moiety of curcumin forms chelates with transition metals, thereby reducing the metal induced toxicity and some of the metal complexes exhibit improved antioxidant activity as enzyme mimics. New analogues with improved activity are being developed with modifications on specific functional groups of curcumin. The physico-chemical and structural features associated with some of the biological activities of curcumin and important analogues have been summarized quite recently [for review see [1, 30, 31].

In a majority of publication that have reviewed the importance of curcumin, special emphasis have been put on the highly promising medicinal applications of metal curcumin complexes, with the three most important areas being anticancer activity, anti-Alzheimer's disease activity and antioxidative/neuroprotective effects [32-35].

Curcumin-metal complexes not only modify the physico-chemical properties of curcumin but they also affect the biological reactivity of the metals. From our proper observations the pro-autophagic and pro-apoptotic activities [11, 36] of curcumin is totally abolished by the complexation (Petit PX, unpublished results). The complexation with curcumin reduces the toxicity of the metals and some curcumin complexes with metals like Cu^{2+}, Mn^{2+}, behave as new antioxidants [14, 15, 17, 18, 29, 37, 38].

Due to the reversible electron transfer reactions with superoxide ions, Cu^{2+} and Mn^{2+}-complexes of curcumin act as superoxide dismutase enzyme mimics. Metal complexes of curcumin have greater significance in view of the pathology of Alzheimer’s disease, where it has been found that due to its lipophilic nature, curcumin can cross the blood brain barrier and chelate metal ions that are toxic to the neurons. It has also been observed that the incidence of Alzheimer’s disease is significantly reduced among people that are known to regularly consume turmeric in their diet. Curcumin forms stable complexes with all the metals involved in Alzheimer’s disease [18, 19, 39]. The interaction of curcumin with Al^{3+}, that has been speculatively supposed to be involved in Alzheimer disease, has been studied extensively. Curcumin forms three different types of complexes with Al^{3+}. In the 1:1

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stoichiometry Al^{3+}-curcumin complex showed less affinity to DNA binding than free Al^{3+}, which has been attributed to its ability to reduce development of Al^{3+}-induced Alzheimer's disease [19, 39]. Many other applications of curcumin have been reported: Ga $^{3+}$ curcumin complexes are being developed as innovative bioceramics [40]. Zn^{2+}-curcumin complexes showed anti-cancer, gastroprotective and antidepressant effects in rats [41, 42]. *In vivo* antiarthritic activity was reported for five co-ordinated curcumin-gold (Au^{3+}) complexes [43]. Vanadyl-curcumin $[VO(Cur)_2]^{2+}$ complexes show antioxidant and anti-rheumatic activities [44]. Through metal coordination, curcumin reduces the toxicity of heavy metals like Hg^{2+}, Cd^{2+}, Pb^{2+} within cell cytoplasm where significant reduction in heavy metal-induced oxidative stress is reported together with complex formation [23, 45-48].

What is interesting here is that curcumin-metal complexes may also exhibit curcumin hormetic behaviour. Whereas, to our knowledge, Curcumin-Fe complexes are unable to induce cell death as curcumin alone did (PXP personal communication).

Curcumin reactivity with ROS

Curcumin with its three reactive functional groups: one diketone moiety, and two phenolic groups [30], substains many chemical reactions which are the hydrogen donation reactions leading to oxidation of curcumin, reversible and irreversible nucleophilic addition reactions, hydrolysis, degradation and enzymatic reactions.

Curcumin has been found to be an excellent scavenger of most ROS (*Figure 6*), a property that referes curcumin antioxidant behaviour in normal cells. ROS consists of both free radical oxidants and molecular oxidants [28, 49-55]

Free radical oxidants participate in electron transfer reactions and in hydrogen abstraction. All three active sites of curcumin can undergo oxidation by electron transfer and hydrogen abstraction. Precise investigations by different groups have confirmed that during free radical reactions, the most easily abstractable hydrogen from curcumin is from the phenol-OH group, resulting in formation of phenoxyl radicals, which are stabilized across the keto-enol structure.

For example peroxyl radicals could reacts with curcumin and give rise to curcumin phenoxyl radicals, which are less reactive than the peroxyl radicals and thereby enhance protection from ROS-induced oxidative stress [28, 50, 52]. The regeneration reaction of phenoxyl radicals back to curcumin by water soluble antioxidants like ascorbic acid, impart the molecule with a chain breaking antioxidant ability like vitamin E [52]. Many other scavenging reactions of several other free radical ROS such as hydroxyl radicals, superoxide radicals and alkoxy radicals by curcumin have been described [49, 51-53]. The reaction of curcumin with superoxide radicals (generally produced at the inner mitochondrial membrane and not very diffusible) has been found to be as efficient as well known lipid soluble antioxidants and the reaction also leads to catalytic degradation of superoxide in which curcumin acts as a superoxide dismutase mimic [51].

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Among the molecular oxidants, reactions with peroxynitrite, hydrogen peroxide are the most common ones. In several biological models curcumin has been found to protect cells under conditions where there is excessive production of these molecular oxidants. However there are not many studies elucidating the possible chemical reactions and identification of the reaction products. It exists few reports in the literature on direct reaction of curcumin with peroxynitrite [54, 55].

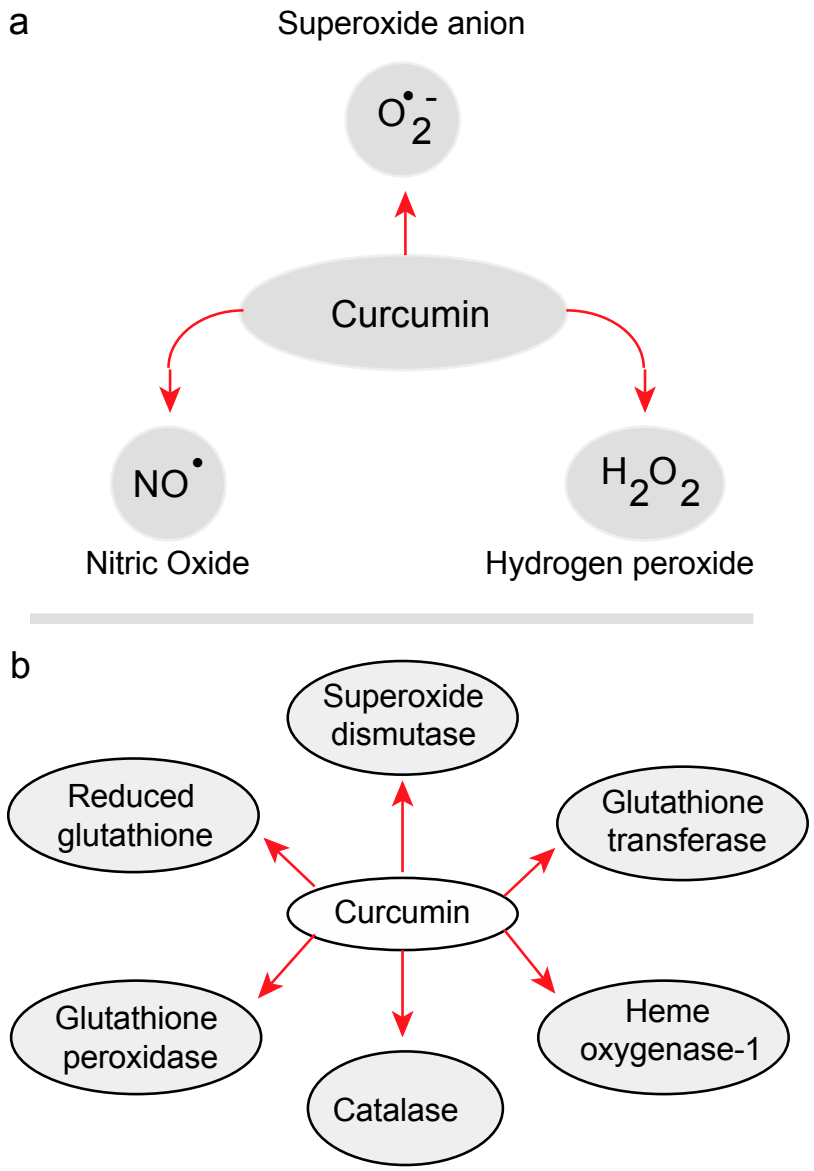

Figure 6

The ROS behaviour and function are interestingly bizarre as a paradoxical main regulator in determining both autophagy, cell survival and cell apoptosis [56]. Up to now, there was no convincing precise explanations regarding curcumin's effects on cancer cells in relation to its anti-oxidant or pro-oxidant functions.

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As discussed above, the diketo functional group in the molecular structure of curcumin is the key active site involved in a plethora of biological reactions, that highligth both anti-oxidant and pro-oxidant capabilities of the molecule [1]. Although the anti-oxidant action of curcumin in normal cells and its diametrically opposed pro-oxidant function in cancer cell has been determined, the precise mechanism of such a dual function, i.e., the biological relevance concerning the chemistry of curcumin, remains quite elusive [1]. Nevertheless, curcumin that has been widely demonstrated as being selective to cancer cell death and enhancing normal cell survival suggests its potential utility as an adjuvant in many chemotherapy and radiotherapy regimens to accomplish enhanced cancer sensitivity and reduced damage to normal tissues [57-59].

Curcumin treatment selectively inhibits cell proliferation in both primary tissue-derived and metastatic tissue-derived cervical cancer cells, while not affecting normal epithelial cells and peripheral blood mononuclear cells. A set of proteins involved in ER-stressed mediated apoptosis is believed to be involved in the curcumin-mediated cell death. It was shown that curcumin induces ER destabilization by insertion in ER membranes, inducing Ca^{2+} release, activating downstream signaling proteins, such as C/EBP homologous protein (CHOP), and three ER transmembrane proteins (PERK, IRE-1α, ATF6), and upregulates the proapototic protein Bcl-2 in cancer cells. These many proteins are usually mediators of ER homeostasis, compatible with cell survival, while excessive accumulation of misfolded proteins in the ER activates apoptosis [60, 61]. So, curcumin over a certain threshold induces an ER stress-mediated apoptosis in cancer cells, while lower levels of ROS generation though the same pathway might result in ER homeostasis, resulting in cancer cell survival under moderate stress like hypoxia, and the evasion of normal cells from apoptosis [62].

Since cancer cells generally maintain high level of ROS, as a consequence of high ROS production or a decline of ROS scavenging capacity, they are selectively vulnerable to further ROS augmentation caused by an exogenous agent like curcumin, a reason for curcumin's selective antiproliferative effect on cancer cells [63]. Enhanced oxidative stress and ROS sensitivity of curcumin-treated cancer cells could also be attributed to the depletion of a reduced thioredoxin (Trx-SH) reservoir caused by the inhibition of thioredoxin reductase 1 (TrxR1) activity [64]. TrxR1 is responsible for Trx-SH turnover from the oxidized Trx-S-S-Trx dimer, which is consumed in the recovery of intracellular SH-proteins. It has been reported that the intracellular free thiol pool defends against the oxidative stresses by reducing disulfide bonds in intracellular proteins, since Trx-SH protein and TRxR1 are the key mediators in the maintenance of redox homeostasis of cells [65].

Curcumin at the cross-road of autophagy and apoptosis

• *Curcumin and mitochondrial biogenesis*

Mitochondria are indispensable for energy metabolism, apoptosis regulation and cell signaling. Mitochondria in malignant cells differ structurally and functionally

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from those in normal cells and participate actively in metabolic reprogramming and are characterized by reactive oxygen species (ROS) overproduction, which promotes cancer development by inducing genomic instability, modifying gene expression and participating in signaling pathways. Mitochondrial and nuclear DNA mutations caused by oxidative damage that impairs the oxidative phosphorylation process will result in further mitochondrial ROS production, completing the "vicious cycle" between mitochondria, ROS, genomic instability and cancer development.

In this general context, mitochondrial biogenesis, i.e. increase of the mitochondrial network, is a complex event depending on both mitochondrial and nuclear genomes to occur in mammalian cells. Mitochondrial biogenesis is usually stimulated under increased energetic demand through a signaling pathway that involves peroxisome proliferator-activated receptor γ co-activator 1-α (PGC-1α) and the nuclear respiratory factors 1 and 2 (NRF1 and NRF2, respectively) [66]. Increased expression levels of PGC-1α is usually found in tissues with high rates of oxidative phosphorylation facing increased ATP needs, such as brain, heart, and skeletal muscle [67-69]. PGC-1α acts upstream of NRF1 and NRF2, activating these transcription factors, in addition to upregulating the estrogen-related receptor α (ERRα) [70-72], leading to an increase expression of nuclear DNA codifying mitochondrial proteins [73]. The expression of the mitochondrial transcription factor A (TFAM) and mitochondrial transcription factors B1 and B2 (TFB1M and TFB2M) is augmented, triggering the expression of specific mitochondrial RNA associated with mitochondrial biogenesis. TFAM is involved in the transcription and replication of mitochondrial DNA (mtDNA) [74, 75] and involves in mtDNA homeostasis [75-77]. PGC-1α is a target of the NAD^+-dependent deacetylase sirtuin 1 (SIRT1) during the control of mitochondrial biogenesis [78-80]. Furthermore, it exists a relationship between AMP-activated protein kinase (AMPK) that is able to modulate the levels of NAD^+ and cause SIRT1 activation (SIRT1 activates PGC-1α through deacetylation) [81]. In this context, the AMPK/SIRT1/PGC-1α signaling pathway orchestrates mitochondrial function and dynamics and participates to the homeostasis of the redox environment.

It is interesting to notice that impaired mitochondrial biogenesis has been observed in human diseases, such as Parkinson's disease [82, 83], Alzheimer's disease [84], cardiovascular diseases [85], and type II diabetes [86].

Curcumin (or new curcumin derivatives) is a pro-apoptotic agent that acts through mitochondria-dependent and independent mechanisms inducing cell death in a wide range of cell types [87-91]. Furthermore, curcumin exerts antioxidant effects upon mitochondria of mammalian cells through different mechanisms, including decreased production of reactive species and upregulation of antioxidant enzymes [92-95]. However, the exact mechanism by which curcumin exerts this effect remains to be completely understood.

The studies demonstrating the role of curcumin as an inducer of mitochondrial biogenesis are just beginning and stay on top of an new avenue for curcumin studies, but might serve as a star-up to develop new drugs that would be utilized in

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pathologies involving mitochondrial dysfunction, such as cardiovascular diseases and neurodegeneration.

It is important to notice that the curcumin external concentrations used in the experimental models that will be discussed here are very high and may not be reached by humans using curcumin contained in food. It will be necessary to impove curcumin bioavailability, to ensure that at e higher curcumin concentrations can be reached in certain target cells and may depend on biotechnology-related strategies considering more permeant curcumin derivatives with a higher intracellular stability [96] also new nanoformulations [96-98]

With *in vitro* experimental models the situation is complex since only few reports are available. Curcumin can induce brown fat-like phenotype in 3T3-L1 and primary white adipocytes [99]. Indeed, curcumin (1–20 μM, 6–8 days) was shown to upregulated PGC-1α. Furthermore, curcumin augmented the levels of cytochrome *c* and of the phosphorylated form of AMPK (p-AMPK) in these cells. Therefore, curcumin can induced an increase of the number of mitochondria through the activation of AMPK/PGC-1α pathway in adipocytes. These data are reinforced by the quantification of mtDNA, as well as the investigation regarding the involvement of other regulators of mitochondrial biogenesis, such as the NRF1 and TFAM.

Curcumin (30 μM as external concentration for 24 h treatment) was shown to induce nuclear factor erythroid 2-related factor 2 (Nrf2) translocation to the nucleus and restore PGC-1α levels in gentamicin-treated LLC-PK1 cells (kidney cell line). It is very likely that curcumin is capable of eliciting mitochondrial biogenesis *in vitro*, since curcumin also increased the number of mitochondria *in vivo* (as it will be discussed below). Nrf2 being is a master regulator of the redox cellular environment [100] and may also play a role in the modulation of signaling pathways associated with mitochondrial biogenesis *in vitro* [101].

Concerning *in vivo* experimental models more informations is accessible. In gastrocnemius and soleus muscles of rats submitted to endurance exercise training [102]. It was found that curcumin induces an increase in the amounts of cytochrome *c* oxidase subunit IV, complex I subunit NDUFB8, complex II 30 kDa subunit, and complex III subunit Core 2. Apparently, Curcumin potentiated the effects of training regarding the upregulation of the respiratory chain components. Curcumin also amplified the effects of exercise training upon mitochondrial DNA (mtDNA) and citrate synthase (CS) activity which is a gold standard of mitochondrial biogenesis in both muscles, demonstrating the ability of curcumin to induce mitochondrial biogenesis *in vivo*. Since AMPK exhibits a central role in regulating mitochondrial biogenesis during energy deprivation events [103, 104], the fact curcumin also activates AMPK is of great interest. The NAD^+-dependent protein deacetylase sirtuin-1 (SIRT1) protein levels also increased in this context [102]. The increase in the NAD^+/NADH ratio reinforces the fact that curcumin activated SIRT1 expression. Additionally, the deacetylation of PGC-1α, a target of SIRT1 during mitochondrial biogenesis [105], was also increased by curcumin in that protocol. Therefore, it has been demonstrated that curcumin activates the AMPK/SIRT1/PGC-1α signaling

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pathway, leading to increased mtDNA and augmented activity of CS (a gold standard for an increase in mitochondrial membrane mass), as well as to an upregulation the respiratory chain componets. It is possible that curcumin elicited alterations in the levels of adenosine monophosphate (AMP) or NAD^+ which then activates AMPK and the signaling pathway leading to mitochondrial bio-genesis.

In rats treated with curcumin and submitted to ischemic reperfusion injury, Liu *et al.* found increased number of mitochondria in the cerebral cortex [106]. Curcumin pretreatment also prevented the injury-induced downregulation of uncoupling protein 2 (UCP2) levels. Moreover, NRF-1 and TFAM, which are responsible for mitochondrial biogenesis [107] and maintenance of mtDNA copy number [108], respectively, were upregulated by curcuminat any dose tested.

Curcumin (400 mg/kg day^{-1} administrated through gavage 5 days prior and 7 days during exposure to gentamicin) allowed preservation of mitochondrial morphology and increased the number of mitochondria in rats exposed to gentamicin, a renal toxin [101]. Curcumin also alleviated the effects of gentamicin regarding oxygen consumption in the presence of malate/glutamate or succinate. Curcumin also restored state 3 and the respiratory control index of mitochondria isolated from gentamicin-treated rats, as well as complex I and complex IV activities. Curcumin caused a delay in mitochondrial permeability transition (MPT) and in the loss of mitochondrial membrane potential (MMP) induced by Ca^{2+} ions *ex vivo*. Even if, the mechanisms underlying the increase in the number of mitochondria was not considered in the *in vivo* experimental model, it was demonstrated that curcumin administered at 30 μM (external concentration) for 24 h before gentamicin exposure allowed the upregulation of Nrf2 and PGC-1α in LLC-PK1 cells (porcine renal epithelial cell line). Clearly, Nrf2 plays a role during mitochondrial biogenesis by upregulating NRF1 in a mechanism associated to the activation of PGC-1α [109-111].

A more recent work demonstrated browning of white adipose tissues in mice treated with curcumin (50 or 100 mg/kg day−1 for 50 days administrated through gavage) [111]. In this case, curcumin increased mtDNA copy number and upregulated UCP1 and PGC-1α in the inguinal adipose tissues.

All this reenforced the new hypothesis that curcumin at very low doses may induce mitochondrial biogenesis even if complementtary studies are needed. Of course, new experimetal data aiming at observing the impact of curcumin upon other regulators of mitochondrial biogenesis, such as AMPK, SIRT1, NRF1, and TFAM will be of great interest.

It is of importance to consider that the following paragraph will try to depict the mechanistic aspects of curcumin-induced autophagy and/or apoptosis but that these events occure in the general context of a very complex intracellular machinery dysregulation. This approach is some how absurd but clearly facilitates the depicting of parallel signaling pathways which all possesed there proper threshold towards i.e., cell cycle arrest, mitochondrial biogenesis, autophagy induction and subsequent autophagic cell death and/ or apoptosis.

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As a matter of fact, in addition to inducing mitochondrial biogenesis, curcumin is able to trigger mitophagy, i.e., the destruction of mitochondria through autophagy-related signaling pathways that might be successful and if not induce cell death.

Curcumin and Its Epigenetic actions on MicroRNA

Recent studies have revealed that curcumin may affect cancer initiation and progression through regulating microRNAs (miRs). miR-21 mediates various effects of curcumin on cancer cells including proliferation, apoptosis, metastasis and anti-cancer drug resistance [112, 113]. Several downstream pathways of miR-21 have been identified including phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), programmed cell death protein 4 (PDCD4) and MAPK pathways, enhancement of p53 and NF-κB pathways. It is interesting to notice that all these pathways have been precedently described as being affected by curcumin cellular loading. Curcumin decreases miR-21 levels by both increasing miR-21 exosome exclusion from the cells and inhibiting the transcription of the miR-21 gene in the cells by binding to its promoter. Beyond miR21 inhibition, curcumin-induced epigenetic alterations consist in the modulation of the expression of several other oncogenic and tumor suppressor microRNAs (miRNAs) (*Table 1*). Suppression of oncomiRs such as miR-21, miR-17-5p, miR-20a, and miR-27a, miR-186* [112] and over-expression of miR-34 a/c and epithelial-mesenchymal transition-suppressor miRNAs are among the most important effects of curcumin on miRNA homeostasis [114].

Table 2 - *Curcumin alters miRNAs and relevant target expression in pancreatic, colorectal, breast and lung cancer*

Cancer origin	Upregulated	Down regulated	Targets	Refs
Pancreas	miR-22 miR200	miR-21 miR-199a*	SP1, ESR1 PTEN	[115] [116]
Colorectum		miR-21	AP1, Pdcd4	[117]
Breast	miR-15a miR-16		Bcl-2	[118]
Lung	miR-186*		Caspase-10	[112]

Abbreviations used : AP1, Activator protein 1 (AP-1) is a transcription factor that regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, and bacterial and viral infections. AP-1 controls a number of cellular processes including differentiation, proliferation, and apoptosis.; Bcl-2 ; Is the protypical member of the Bcl-2 protein family that inhibit apoptosis ; Caspase-10, This protein cleaves and activates caspases 3 and 7, and the protein itself is processed by caspase 8. Mutations in this gene are associated with apoptosis defects seen in type II autoimmune lymphoproliferative syndrome. ; ESR1, Estrogen Receptor 1; Pcdc4, Programmed cell death protein 4 is a protein that in humans is

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encoded by the *PDCD4* gene. It is one of the targets of an oncomiR or miR-21; PTEN, This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin-like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. ; SP1, The protein encoded by this gene is a zinc finger transcription factor that binds to GC-rich motifs of many promoters. The encoded protein is involved in many cellular processes, including cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage, and chromatin remodeling. Post-translational modifications such as phosphorylation, acetylation, glycosylation, and proteolytic processing significantly affect the activity of this protein, which can be an activator or a repressor.

Curcumin at the cross-road between autophagy (i.e. mitophagy) necroptosis and apoptosis

Interconnections between apoptotic, autophagic and necrotic pathways are very numerous which render separate pathway analysis difficult. Cell death plays an essential role in the development of organs, homeostasis, and cancer. Apoptosis and programmed necrosis are two major types of cell death, characterized by different cell morphology and pathways. Accumulating evidence shows autophagy as a new alternative target to treat tumor resistance. Besides its well-known pro-survival role, autophagy can be a physiological cell death process linking apoptosis and programmed necrosis cell death pathways, by various molecular mediators.

Curcumin and autophagy

Autophagy, is a process of cytoplasm and cellular organelle degradation in lysosomes implicated in homeostasis, thus functioning as an important biological mechanism in targeting human cancers. Curcumin acts as a double-edged sword as both a tumor suppressor and a cancer cell survival protector [119-122]. The accumulation of autophagosome in dying cells is highly correlated with the autophagic cell death (which is also defined as a non-apoptotic form of programmed cell death (PCD) or type II PCD) with possess a potential function of tumor suppression similar to apoptosis. With the discovery and characterization of Atg, the suppressive function of autophagy in cancer has been validated [123, 124]. Among a series of Atg genes, Beclin 1 (Atg6) is an essential tumor suppressor that modulates the initiation and regulation of autophagy. The high frequent allelic BECN1 deletion is often present in human breast, ovarian and prostate cancers and aging Becn1$^{+/-}$mice are prone to tumors including lymphomas, lung and liver cancers [125-127].

On the other hand, autophagy has a tumor suppressive role since the cell survival function of autophagy during cancer progression under stress environments has been validated. For example in immortalized, apoptosis-defective, IL-3-dependent bone marrow cells in response to growth factor deprivation could result in the extension of cell survival due to the autophagy induction; correspondingly, the

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accelerative cell death in the presence of autophagy inhibition has also been confirmed [128, 129]. Moreover, increasing evidence has clearly documented that autophagy in cancer cells is up-regulated in response to metabolic and genotoxic stress induced by hormonal deprivation, chemotherapy and radiation as a cell survival mechanism, thereby contributing to treatment resistance [119]. Furthermore, the alteration of autophagic flux may be highly correlated with the cell or tissue type, correspondingly determining the cell fate such as cell survival and cell death under stress conditions.

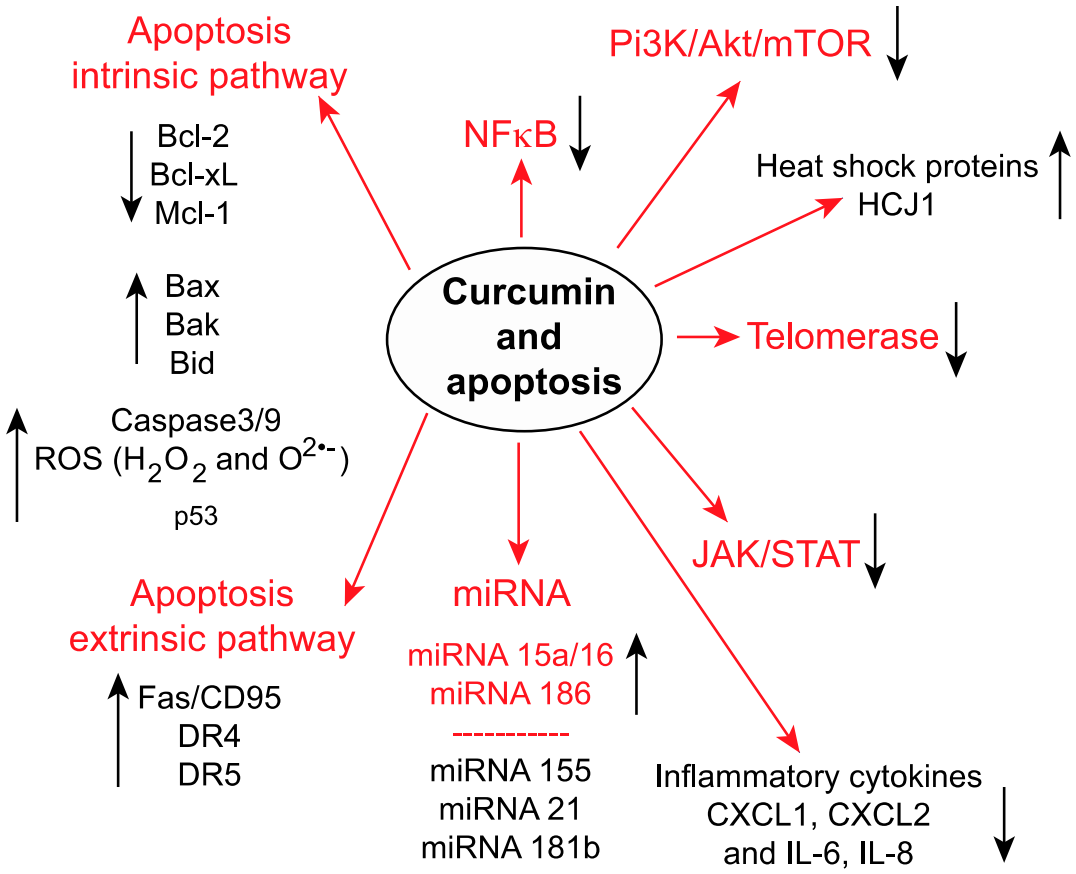

Figure 7

The inflammation occurrence and vascular insufficiency in tumors can lead to the depletion of glucose and/or oxygen, thus perturbing the osmotic milieu causing extracellular acidosis in tumor microenvironment and eventually inducing the autophagy. Therefore, the functional status of autophagy will in govern tumor metastasis and subsequent carcinogenesis or be a determinant for the therapeutic strategies of cancers [119, 120]. Targeting or manipulating autophagic signaling pathways may be an innovative strategy during the exploration cancer-associated

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biomarkers, and the prevention and treatments or even combinatorial treatments of cancers in the near future. .

In this context, curcumin has a wide range of biological functions, especially the anticancer activity that can be exploited. Curminin can inhibit the proliferation of tumor cells (*Table 1*), and induce the apoptosis of tumor cells (*Figure 7*) including bladder cancer [130], pancreatic cancer [131], prostate cancer [132] and uterine cervix carcinoma [133]. Curcumin also exhibits favorable synergistic performance both in thermotherapy and γ-ray therapy for cancers [134, 135]. The injection of curcumin in mice bearing breast cancer accomplishes an obvious inhibitory effect on the growth of breast cancer cells [136].

Curcumin has also been found to greatly inhibit the metastasis of breast cancer cells. Previous reports have revealed that curcumin can inhibit cell proliferation of chronic granulocytic leukemia (CGL), glioblastoma, and esophageal cancer through inducing autophagy. It can also inhibit the growth of leukemia K562 cells accompanying with the up-regulation of LC3-II and Beclin 1 as well as the accumulation of autophagosomes. In contrast, in the presence of autophagy inhibitor Bafilomycin A1, the curcumin-induced death of K562 cells is obviously inhibited, suggesting that curcumin can induce autophagy and K562 cell death. In glioblastoma cells, curcumin can inhibit Akt/p70S6K signal pathway, activate extracellular signal-regulated kinases (ERK1/2), and finally induce autophagy [137]. It can induce the generation of reactive oxygen species (ROS), up-regulate the expression of Beclin 1 and p53, activate autophagy, and eventually result in the death of human colon cancer cells. Serine/threonine protein phosphatases type-1 (PP1) and PP2A are key targets of phosphorylation of ERK, and curcumin can stimulate the phosphorylation of ERK by inhibiting PP1 [138]. Besides activating autophagy, curcumin also exhibits time- or concentration-dependent inhibition on the growth of K562 cells. Curcumin-induced cell death is highly correlated with the generation of apoptotic or autophagy complexes, mitochondrial membrane potential (MMP) and the activation of caspase-3. In addition, curcumin can cause the down-regulated expression of Bcl-2 protein in K562 cells [139]. The combinatorial treatment of curcumin and adriamycin on human Hepatoma G2 (HepG2) facilitates to the apoptosis of HepG2 cells due to the reduced proportion of Bcl-2/Bax protein and caspase-3 activation. Moreover, curcumin can result in the mitochondrial fission of HepG2 cells, reduced potential of mitochondrial membrane and autophagy activation. These results have shown that curcumin is likely to strengthen adriamycin-induced HepG2 cell death rate through activating mitochondria-mediated autophagy [140].

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Table 2- *Antiproliferative target for curcumin*

Target	Effect	Cancer type	refs
GRP78	Downregulation	Colon	[141]
EphA2	Downregulation	Melanoma	[142]
SOCS 1&3	Upregulation	Leukemia	[143]
Nfr2	Downregulation	Breast	[144]
MiR-15a/16-1	Downregulation	Leukemia	[145]
DCLE1	Upregulation	Colon	[146]
Skp2	Downregulation	Glioma	[147]
FOXO1	Upregulation	Pancreas	[148]
EZH2	Downregulation	Breast	[149]

Abbreviation used : EphA2, EPH receptor A2 (ephrin type-A receptor 2) is a protein that is encoded by the *EPHA2* gene and belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family ; EZH2, Enhancer of zeste homolog 2 is a histone-lysine N-methyltransferase enzyme (EC 2.1.1.43) encoded by EZH2 gene, that participates in DNA methylation and, ultimately, transcriptional repression ; FOXO1, Forkhead box protein O1 also known as forkhead in rhabdomyosarcoma is a protein that in humans is encoded by the *FOXO1* gene. FOXO1 is a transcription factor that plays important roles in regulation of gluconeogenesis and glycogenolysis by insulin signaling, and is also central to the decision for a preadipocyte to commit to adipogenesis. It is primarily regulated through phosphorylation on multiple residues; its transcriptional activity is dependent on its phosphorylation state; GRP78, is a member of the HSP family of molecular chaperones required for endoplasmic reticulum integrity and stress-induced autophagy which plays a central role in regulating the unfolded protein response (UPR), and is an obligatory component of autophagy in mammalian cells; Nfr2, Nuclear factor (erythroid-derived 2)-like 2, also known as NFE2L2. Nrf2 is a basic leucine zipper (bZIP) protein that regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation; Skp2, S-phase kinase-associated protein 2 ; SOCS 1&3 These genes encodes members of the STAT-induced STAT inhibitor family (SSI), also known as suppressor of cytokine signaling (SOCS).

Curcumin, ER-mitochondria and apoptosis

The initial effects of curcumin could primarily be due to its interaction with subcellular compartments, as toxicity of such polyphenol compound slighthly lipophillic could be associated to total lipophilic load. The endoplasmic reticulum or the lysosomes are examples of such subcellular compartments, since oxidative stress [150-153], lipid peroxidation [154] and calcium raise [155, 156] have been shown to be associated to curcumin treatments and all these events are involved in death induction [152, 157] or more precisely apoptosis [158-160].

A plethora of publications proved that curcumin can bind and/or inhibit numerous targets including, for example, Nrf2, β-catenin, NF-κB, inducible nitric

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oxide synthase, nitric oxide, amyloid plaques, reactive oxygen species (ROS), cyclin D1, glutathione, cytosolic phospholipase A2, inhibitor of NF-κB kinase-1-2, P38MAPK, p-Tau (p-τ) and tumor necrosis factor-α.

Several mechanisms by which curcumin exerts its anticancer effect have been reported most of them are more or less related to cell death induction. Effectively, curcumin inhibits a transcription factor, nuclear factor κB (NF-κB), by inhibiting inhibitor of κB kinase and subsequent IκBα phosphorylation [161-164]; As a result, curcumin down-regulates the expression of NF-κB-regulated gene products such as Bcl-2, Bcl-XL, cyclin D1, cyclin B1, matrix metalloproteinase-9, cyclooxygenase-2, and interleukin-6, resulting in cell cycle arrest (G2/M) (*Figure 8*), suppression of proliferation, and induction of apoptosis [161, 162, 165, 166]. Second, curcumin inhibits the Akt/mammalian target of rapamycin (mTOR) pathway and phosphorylation of p70 ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein, resulting in inhibition of proliferation and induction of apoptosis [162, 167-169]. Other mechanisms of the antitumor effect of curcumin include down-regulation of transcription factors activator protein-1 [170-174] and Egr-1 (*Figure 8*).

Curcumin also inhibit tumor cell growth and suppress cellular entry of viruses very potently [175], and is efficient in suppressing phorbol-ester-induced tumor promotion [176]. Curcumin insertion into intracellular membranes affects membrane mobility and permeability, also acting on ion channels and transporters. But the main characteristics of curcumin, justifying its hormesis capability, is to possess both antioxidant and pro-oxidant properties that are closely related to autophagic and cell death activation processes [36].

Molecular circuitries that link curcumin to cellular stress and death, and how these pathways can get uncoupled during hormetic responses is a subject of great interest [11, 36]. It is reported that curcumin at very low doses (≤1 μM) is indeed an excellent antioxidant but that medium doses of curcumin (in the range of 5–10 μM) operates primarily as an autophagy inducer, correlating with their described capacity to reduce the acetylation of cytoplasmic proteins and cell cycle blocker (*Figure 8*). Finally, at further higher doses (over 25μM), cell death is induced (all experiments run for 48h). We investigated mechanistic aspects of the destabilization of the endoplasmic reticulum (ER) and lysosome involved in mitochondrially associated apoptosis. Curcumin induces an ER stress causing calcium release which in turn destabilizes the mitochondrial compartment to induce apoptosis. These events are also associated with lysosomal membrane permeabilization and activation of caspase-8, mediated by activation of cathepsins and calpains [11]. This complex interplay is of huge interest, as the efficient autophagy may allow cells to escape the G2/M blockage [11] induced by curcumin when used at 10 μM (*Figure 8*).

Curcumin, lysosomes and autophagy

Autophagy has attracted the interest of scientists in the field of cancer research because it is designated as programmed cell death type II, whereas

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apoptosis is wellknown as programmed cell death type I [177]. But such definition has been modified and adapted to the discovery of multiples cell death pathway as well as the involvement of diverses types autophagy. More precisely, autophagy is a catabolism process through utilizing lysosomes to degrade damaged, denatured and aged proteins and organelles in cells. Under normal physiological conditions, the basal autophagy is a catabolism process through utilizing lysosomes to degrade damaged, denatured and aged proteins and dysfunctionnal organelles in cells.

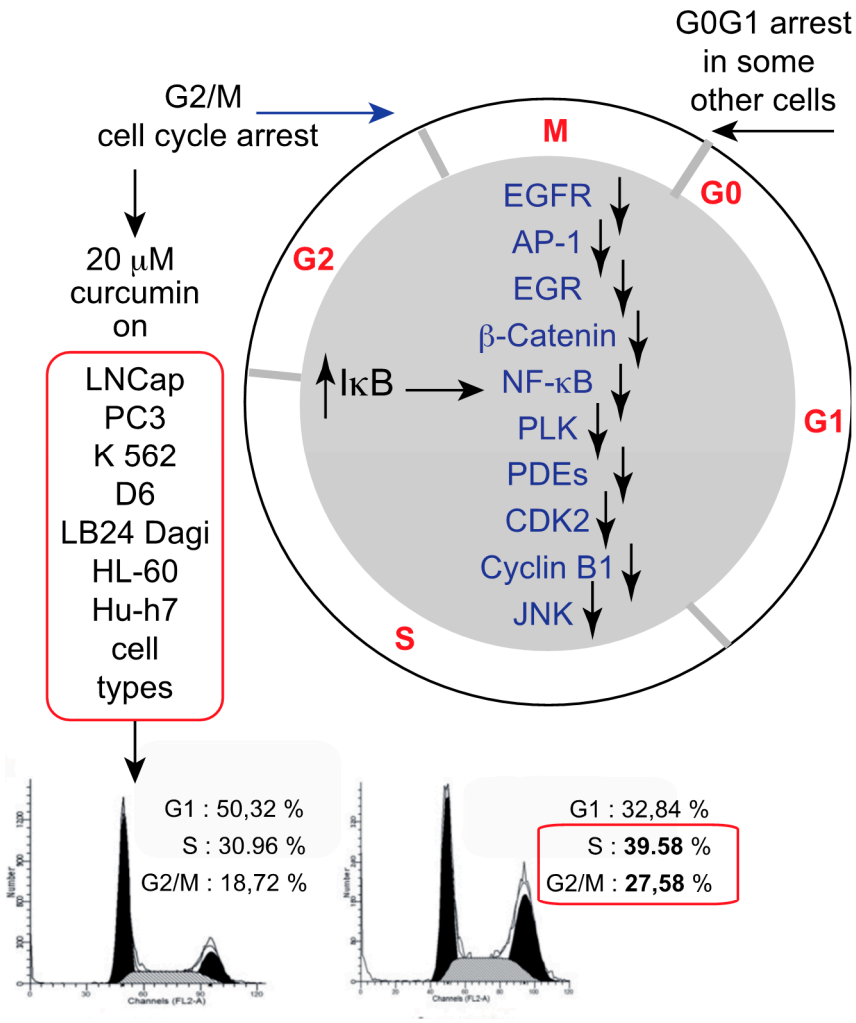

Figure 8

It is very important to notice that a portion of the internalized curcumin is bind to the lysosomal membranes [11] (*Figure 4*). The role of curcumin concerning lysosomal destabilization is critically dependent on the intracellular curcumin

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concentration and the occurrence soluble lysosomal hydrolases (e.g. cathepsins, chemotrypsin) as well as lysosomal membrane proteins dysfunction (e.g. lysosome-associated membrane proteins).

It has been reported that curcumin induce autophagy through inhibition of the Akt-mTOR pathway. There is a growing body of proofs arguing for highly activated PI3K/Akt signalling in cancer cells compared to normal one. Interestingly, curcumin has been found to interfere with PI3K/Akt pathway leading to inhibition of cell proliferation, lowering invasivness and cell migration in various cancer cells including triple negative-cancer cells [178-181].

The Akt/mammalian target of rapamycin (mTOR)/ p70 ribosomal protein S6 kinase (p70S6K) and the extracellular signal-regulated kinases 1/2 (ERK1/2) pathways are two major pathways that regulate autophagy induced by nutrient starvation. These pathways are also frequently associated with on- cogenesis in a variety of cancer cell types, including malignant gliomas

In U87-MG and U373-MG malignant glioma cells, curcumin induced G2/M arrest (*Figure 7*) and non-apoptotic autophagic cell death. It inhibited the Akt/mTOR/p70S6K pathway and activated the ERK1/2 pathwayand induce autophagy. It is interesting that activation of the Akt pathway inhibited curcumin-induced autophagy and cytotoxicity, whereas inhibition of the ERK1/2 pathway inhibited curcumin-induced autophagy and induced apoptosis, thus resulting in enhanced cytotoxicity. These results suggest that curcumin has high anticancer efficacy *in vitro* and *in vivo* by inducing autophagy [138]. Bap31 has been taugth to direct pro-apoptotic crosstalk between the ER and the mitochondria *via* Ca^{2+} in conjunction with caspase-12 and calnexin [182, 183]. Accordingly, ER stress and the resultant Ca^{2+} release must be very carefully regulated because of their effects in virtually all areas of cell function [184]

Nevertheless, the effect of Curcumin on lysosome remains largely elusive. Somes recent data suggested that currently known TFEB activators are mainly inhibitors of mTOR (mechanistic target of rapamycin [a serine/threonine kinase]), which, as a master regulator of cell growth and metabolism, is involved in a wide range of biological functions [185] and exert its function at the lysosomal membrane surface [185].

It has been found that curcumin treatment enhances autophagic fluxes in both human colon cancer HCT116 cells and mouse embryonic fibroblasts (MEFs). Moreover, Curcumin treatment promotes lysosomal function, evidenced by the increased lysosomal acidification and enzyme activity. In the same context, curcumin is able of suppressing the mammalian target of rapamycin (mTOR). It has also been advanced that curcumin treatment may activates transcription factor EB (TFEB) [186]. TFEB is also a major player of the transcriptional response to starvation and controls autophagy by inducing lysosomal biogenesis, regulating autophagosome formation and autophagosome-lysosome fusion both *in vitro* and *in vivo* [187]. This is based on cardinal hypothesis that a lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via TFEB and mTOR [188]. At the lysosomal membranes, the

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Transcription Factor EB (TFEB), a master regulator of lysosomal biogenesis, colocalizes with master growth regulator mTOR complex 1 (mTORC1). The arguments for the fundamental role of TFEB are the following : Curcumin is able directly binds to TFEB (and/or to disturb membrane at the vinicity of TFEB insertion), to promotes TFEB nuclear translocation and increases transcriptional activity of TFEB. Indeed, the discovery of TFEB modulators that acts without inhibiting the mTOR pathway would be probably less deleterious to cells [189]. Along with this new argument, it has been described that curcumin fails to inhibit mTOR and to activate lysosomal function when constitutive activation of mTOR has been ingineered, proving that curcumin-mediated lysosomal activation is achieved via suppression of mTOR. Finally, inhibition autophagic fluxes and activation / destabilization of the lysosomal compartment by curcumin, if passing a certain threshold, leads to more cell death, suggesting that autophagy and lysosomal activation serves as a cell survival mechanism to protect against curcumin-mediated cell death.

Taken together, all these recent data provide a novel insight into the regulatory mechanisms of curcumin on autophagy and lysosomes, which may facilitate the development of curcumin as a potential cancer therapeutic agent [186] but may also be used for lysosomal storage disorders, neurodegenerative disorders, and cardiovascular diseases.

Curcumin and mitophagy

Mitophagy is a conserved, mitochondria-specific autophagic clearance process. Recently an intricate regulatory network that balances mitophagy with mitochondrial biogenesis preserving homeostasis has been highligthed [190]. Mitochondrial biogenesis and mitophagy are two pathways that regulate mitochondrial content and metabolism preserving homeostasis. The tight regulation between these opposing processes is essential for cellular adaptation in response to cellular metabolic state, stress and other intracellular or environmental signals. Proper coordination of these opposing processes is important for stress resistance and longevity. Nodal regulatory factors that contribute to mitochondrial homeostasis have also been linked to carcinogenesis, highlighting mitophagy as a potential target for therapeutic interventions against cancer [191].

More precisely, mitophagy is the process by which damaged mitochondria are removed from the cells through engulfment by an active autophagosome in a PTEN-induced kinase 1 (PINK1)/Parkin (E3 ubiquitin ligase)-dependent mechanism [192, 193]. In spite of being a physiological process, increased rates of mitophagy have been found in some human pathologies and may represent a risk regarding the maintenance of both redox and bioenergetics status in those cases autophagosome [194-197].

Curiously, the capability of curcumin to induce mitochondrial biogenesis (certainely at low doses) to face enhanced energetic demand, is accompagnied curcumin potentiation to trigger mitophagy [11, 198]. Mitophagy is defined as the process by which damaged mitochondria are removed from the cells through

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engulfment by an active autophagosome in a PTEN-induced kinase 1 (PINK1)/E3 ubiquitin ligase (Parkin)-dependent mechanism [192, 199]. In spite of being a physiological process, increased rates of mitophagy have been found in some human pathologies and may represent a risk regarding the maintenance of both redox and bioenergetics status in those cases [194-197].

Thus, the balance between mitochondrial biogenesis and mitophagy is of particular interest regarding pharmacological aspects, because these events represent important targets that may be explored therapeutically in several human diseases. Once again, the notion of sthrehold is evidenced.

It has been demonstrated that curcumin at 10 μM sensitized CNE2 cells (nasopharyngeal carcinoma) exposed to ultrasound [200]. The combination of ultrasound and curcumin increased the number of swollen mitochondria, as well as impaired mitochondrial membrane architecture. This results establish early taugth that altered mitochondria could be eliminated by a specific autophagy (i.e., mitophagy) in presence of curcumin.

Additional data need to be obtained in order to elucidate the exact role of curcumin as an inducer of mitophagy and the circumstances in which this molecule would be useful in inducing mitochondrial degradation.

Conclusion

At the overall, one can say that even if almost a tausend of publications containing the term curcumin (≥ 9666, Pubmed, January 2016), they are only few incomers when it is question of new research directions. It is clear that low level of curcumin is able to elicit mitochondrial biogenesis in cells and tissues mainly through the well-known induction of the PGC-1α-related signaling pathway [201, 202]. The exact mechanism by which curcumin triggered mitochondrial biogenesis remains to be fully understood, since a role of AMPK, NRF1, Nfr2, and/or TFAM that is essential in biogenetic processess was not investigated in most of the studies that where published. Indeed, molecules such as the PGC-1α are though to be critiacl for the maintenance of organelle contnet in a specific tissue-specific manner. The idea that the induction of mitochondrial biogenesis by exogenous compounds may play a role in alleviating cellular dysfunction involving disruption of mitochondrial bioenergetics, such as cases of neurodegenerative and cardiovascular diseases, is of great value. Even if, on the other hand, our knowledge of the regulation of mitochondrial biogenesis induced by curcumin are scarces [75, 76, 77].

The role of curcumin as an inducer of mitochondrial biogenesis as well as of mitophagy in a context of mitochondrial energetic disturbance remains at its infancy. However, with curcumin it could also been usefull to cope with the transcription factor EB (TFEB), the protein widely considered to be the most important regulator of autophagy and lysosomal biogenesis, would shed ligth on the regulation of mitophagy in various cell types.

Curcumin is undoubtly inducing crosstalk between apoptosis and autophagy mutual proteins to regulate cancer cell death. For most of the researchers, the course

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of cancer advancement has always been attributed for a great part to the defectiveness in cell death mechanisms [203, 204]. These defects act as a shield in protecting tumor cells from drugs and therapies, all at the same time, maintaining a longer life span and prompting their dispersion procedures. Autophagy and apoptosis safeguards cells from cellular damages and maintains proliferation and homeostasis by deporting outgrowth and controlling differentiation of pernicious cells. The autophagic proteins are usually found in hindering apoptosis whereas *vice-versa* accounts had been reported for apoptotic-intermediates in preventing autophagic responses.

In case of curcumin, autophagy and apoptosis that are highly intricated pathways can be instigated in a congruous or interdependent manner through manipulations of their mutual proteins. Targeting those mutual proteins that crosstalk between autophagy and apoptosis to regulate tumor cell death is crucial for the successful design of future anticancer therapies. For sure, microRNA (miRNA) are involved in the modulation of these interplaying components that may set off both autophagy and apoptosis in cancer cells. Since, the development of miRNA-based therapeutic alone seem laborious (critical drawbacks) and a long drawn out story. Curcumin as a phytochemicals, may shorted this gap since its capabilities of switching these interplaying proteins to maximize cancer cell death through the partnership of autophagy and apoptosis have been reported [11, 36]. By doing so, we will be able to chart the missing links between these machinery proteins, curcumin chemopreventive properties and miRNAs.

Beyong curcumin, the role of green natural chemopreventive agents like curcumin in autophagy and apoptosis [205] will expand the existing knowledge on miRNAs hence construct a devising road to a tactical anti-cancer therapy with minimized adverse effects [206]. As natural products are an essential source in discovery of lead compounds of anticancer drug, studies on the role of natural product anti-cancer agents that induce cross-talk between apoptosis and autophagy mutual proteins to regulate cancer cell death as a design of future green anticancer therapies is at the forefront of new therapeutic discoveries.

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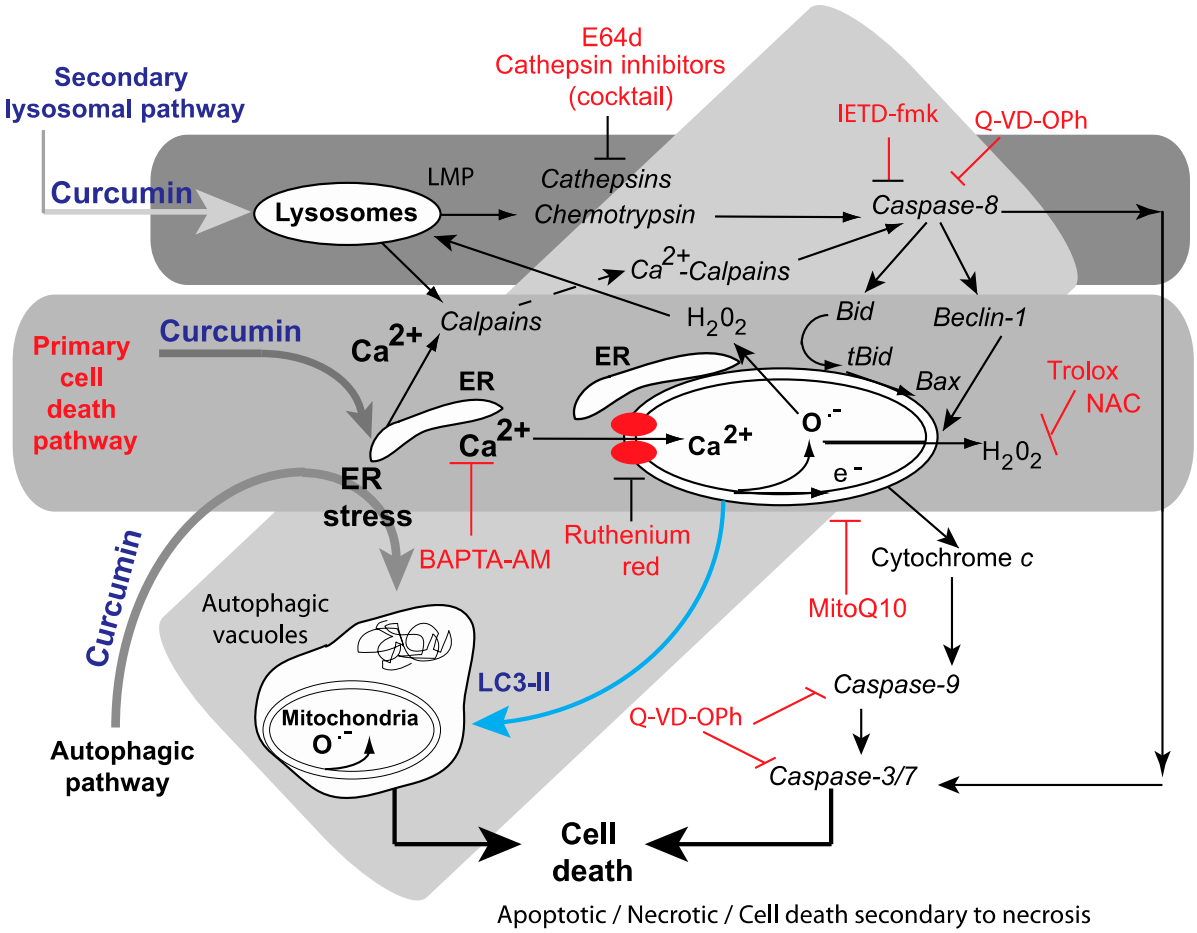

Figure 9

Acknowledgements

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Competing interest

The authors declare no conflict of interest.

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• Legends of Figures

Figure 1 - *Curcumin structure and first degradation products.* This schematic interpretation are alls issued from Priyadarsini K.I. with permission [1].

Figure 2 - *General consideration about the curcumin molecules*

a - Synthesis of curcumin by the general method proposed by Pabon [3].

b - Keto-enol tautomerism, prototropic equilibria and degradation products of curcumin.

c - Possible sites of attack of free radical oxidants, stabilization of phenoxyl intermediate and its regeneration by ascorbic acid.

The schematic interpretation are all extracted from Priyadarsini K.I. with permission [1].

Figure 3 - *Curcumin reactions with ROS (a) and curcumin upregulation of anti-oxidant system compoments (b).* Reprinted and modified from [1].

Figure 4 - *Curcumin also localize at the endoplasmic reticumum membrane.*
[Patrice X. Petit, personal data and [11].

Figure 5 - *Lysosomal localization of curcumin as a function of the concentration*.

The cells are stained with lysotracker red from Molecular probes and curcumin fluorescence is measured in parallel with an AMNIS image-flow cytometer [P.X. Petit, J.-E. O'Connor and F.J. Sala de Oyanguren, original data, not published]. The equation is given for the analysis of co-pixelisation

Figure 6 - *Reaction of curcumin with metals.*

a - Structure of 2:1 curcumin:metal complex.

b - Mixed ligand curcumin:metal complex.

Reprinted and modified from [1] with permission.

Figure 7 - *Schematic interpretation of the effects of curcumin on apoptosis.*

The scheme has been taken from Pavan et al. [207] redrawn and completed. In red color, th emain pathways and proteins affected. In black additives proteins that might be afefcted depending of the tissue.

Figure 8 - *Schematic interpretation of curcumin interaction with the cell cycle and short list of proteins involved.*

Figure 9 - *Crosstalk between apoptosis and autophagy in cells treated by curcumin*.

Curcumin mainly targets the endoplasmic reticumum (ER) and lysosomes. The classic apoptotic pathway is mediated by calcium release from the ER. Uptake of this calcium by mitochondria disrupts mitochondrial homeostasis. Calcium alters mitochondrial electron transport causing substantial ROS production (both

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superoxide anions and hydrogen peroxide), which leads to the opening of the permeability transition pore in the mitochondrial membrane. Consequently, cytochrome c is released and the caspase-9 and caspase-3/7 pathway is activated leading to cell death. Furthermore, the ER stress pathway leads to the formation of autophagic vacuoles that attempt to eliminate the dysfunctional mitochondria. The cleavage of Beclin-1 is associated with early apoptosis and leads to the accumulation of autophagic vacuoles. So, despite the activation of autophagy, cells undergo a type of ‘necrotic cell death’ following these initial apoptotic events. These two pathways are parralled by a lysosomal pathway that is dependent on the curcumin concebtration (see Figure 4). Indeed, curcumin destabilizes lysosomal membranes leading to lysosomal membrane permeability and the activation of both cathepsins and chemotrypsins. Activated caspase-8 leads to Beclin-1 cleavage that inhibits the primarily induced autophagy. The increase in cytosolic calcium concentration also activates calpains that contribute to the degradation process and accelerate cell death. The various inhibitors used in this work are indicated in red at the place where the pathways are affected [11, 36] (Copyrigth Patrice X. Petit). The big gray arrows indicates the entrance of the three main pathways that interfere [the primary cell death pathway, the secondary lysosomal pathway (also promoting cell death) and the autophagic pathway].

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