CASE REPORT

Curcumin-Induced Apoptosis in the Repair of Photodamaged Skin

Madalene CY Heng*

UCLA School of Medicine, USA

*Corresponding author: Madalene CY Heng MD, FRACP, FACP, FAAD, Professor of Medicine/Dermatology/David Geffen UCLA School of Medicine, 500 Paseo Camarillo, suite 100, Camarillo California 93010, USA, E-mail: madaleneheng@aol.com; madalene@madalenehengmd.com

Abstract

Ultraviolet light-induced damage to the DNA resulting in double-stranded triggers the DNA Damage Response (DDR). This results in Cell Cycle Arrest (CCA) and Nucleotide Excision and Repair (NER) of the photo-damaged DNA. These processes are regulated by a family of phosphatidylinositol-3-kinase family. Cell cycle arrest is inhibitory to rapid healing of tissues. When damage is severe, apoptosis is induced only after multiple failed attempts at repair. Moreover, the intrinsic repair processes are slow and laborious, and do not return the tissues to pre-injury levels. When large segments of the DNA are damaged, or if the damage involves both strands of the DNA, replication errors occur, producing a potentially premalignant cell. The activation of the DDR pathway is regulated by a family of phosphatidylinositol-3-kinase family, the phosphorylation of which may be enhanced by phosphorylase kinase. It is proposed that by avoiding activation of the DDR pathways through inhibition of phosphorylase kinase, curcumin may induce early apoptosis and avoid sequestration of the healing process in “Cell Cycle Arrest” The advantages of early apoptosis allows not only for removal of damaged cells, but prevents the survival of potentially premalignant cells in burns and premalignant lesions in photo-damaged skin. The replacement of damaged cells by new healthy cells allows for more complete repair process than would otherwise occur.

Introduction

Ultraviolet light-induced DNA damage results in helix distorting DNA lesions predominantly consisting of cyclobutane pyrimidine dimers (CPDs) and 6-4-photoproducts (6-4PP) [1-4]. The damaged DNA segments are removed by nucleotide excision repair (NER) that eliminates large segments of helix distorting lesions in a multistep “cut and patch” type reaction [5]. The damaged DNA is excised as a 25-30 oligonucleotide DNA fragment followed by gap filling through DNA repair synthesis and restoration of an intact DNA duplex by a final ligation step.

Cells activate distinct signaling pathways, in particular, the DNA damage response (DDR pathway, in response to various types of DNA damage, in particular to double stranded DNA breaks (DSBs). The recognition of these breaks is achieved by the multifunctional sensor complex, MRN [5] (MRE11-RAD50-NBS1) that consists of the structure-specific nuclease (MRE11; meiotic recombination 11 protein complex), the ATPase and adenylate kinase subunit (RAD50; radiation sensitive DNA repair protein 50), and the adaptor protein (NSB1; Nijmegen syndrome breakage 1). MRN complexes can bind to the exposed DNA ends directly and unwind the DNA ends in an ATP-dependent manner. One or the earliest events in DDR is the recruitment of the phosphatidylinositol 3-kinase-like kinase (PI-3KK), ATM kinase, by the MRN sensor complex [5-9]. This leads to the dissociation of the inactive dimer, and activation of ATM kinase, a key early enzyme in the DDR pathway.

The presence of double stranded DNA breaks (DSB) following ultraviolet injury triggers the DNA damage response (DDR) signaling pathway (Figure 1), through activation of ATM kinase, resulting in nucleotide excision and repair (NER) through histone (H2A) ubiquitination [5,10]. ATM (ataxia telangiectasia mutated) kinase, a member of the phosphatidylinositol 3-kinase family, is responsible for phosphorylating H2AX at ser193 (γH2AX) in response to DSBs [10]. In chronic ultraviolet injury, the ultraviolet-induced replication stress activates ATR (ataxia telangiectasia rad 3 related) kinase, another member of the phosphatidylinositol 3-kinase family, resulting in the recruitment of ATR kinase by ATR-interacting protein to replication protein A (RPA)-coated single stranded DNA, which occurs at stalled replication forks [11]. Both ultraviolet damage and ultraviolet rep-
multiple sites triggers ubiquitylation signals [19]. In addition, certain reactions can be bypassed. For example, DNA-PK is able to regulate H2AX phosphorylation in response to DNA damage by bypassing ATM-dependent phosphorylation [20].

To ensure genomic integrity, cells have developed DDR pathways that sense DNA damage, and catalyze a multifaceted response that coordinates cell cycle arrest, apoptosis and DNA repair networks. The regulation of cell cycle checkpoints and DNA repair pathways are strictly coordinated and under tight control to achieve accurate DNA repair and prevent the introduction of errors. The genome is particularly vulnerable during DNA replication. If not properly repaired, cell can undergo apoptosis or senescence. Alternatively, dysregulation or impaired function of proteins involved in the improper repair during the DDR can result in chromosomal changes, genomic instability and increased cancer risk. The DDR pathways which result in apoptosis, cell cycle arrest, and DNA repair pathways are summarized in Figure 2.

Apoptosis and the Decision Process: To Die or Not to Die

In the case of severe irreparable DNA damage, apoptosis is induced. The intrinsic mechanism involves activation of caspases, and it is currently believed that pro- and anti-apoptotic pathways from environmental cues regulate the activation of caspases. The serine/threonine binding protein, 14-3-3, possesses a diverse array of client proteins and is thought to be responsible for coordinating survival and apoptotic pathways through this array of binding partner proteins [21,22]. However, the exact mechanisms for the decision making process of whether “to die” or “not to die” is still uncertain [21]. and many reactions can result in both cell survival and apoptosis depending on the circumstances.

Cells have developed checkpoint proteins to coordinate complex regulatory networks to sense DNA damage, coordinate DNA replication, cell-cycle arrest and DNA repair. The histone protein, H2AX, specifically controls recruitment of DNA repair proteins to sites of DNA damage. The MDC1 (mediator of CHK1) works with H2AX to promote recruitment of repair proteins to sites of DNA breaks, as well as induce cell-cycle arrest checkpoints [18]. Although it is known that these interactions are achieved in a phosphorylation-dependent manner, the exact protein kinase(s) responsible for these interactions are still uncertain. It has been proposed, however, that phosphorylation at

Figure 1: Recruitment of repair proteins to sites of DNA damage (double stranded breaks).
Additionally, genotoxic stress caused by double-stranded DNA breaks may also induce apoptosis via NF-κB dependent pathways [26]. The role of genotoxic stress (double stranded DNA breaks) in the induction of PIDD (p53 inducible death domain containing protein) has been studied by Janssens, et al. [27] with PIDD being responsible for activation of NF-κB-dependent apoptosis via caspase 2 activation (Figure 2).

It must also be pointed out that 14-3-3 proteins are better known for their promotion of cell proliferative or survival functions. Thus, members of the Raf family (MAP kinase, kinase, kinase), which are serine/threonine kinases, are key activators of the Ras GTPase that initiate the activation of the extracellular-regulated kinase (ERK) or MAP kinase, a tyrosine kinase, which promote cell proliferation and survival. These kinases, activated by NF-κB-dependent pathways, form a parallel pathway which are anti-apoptotic, and promote cell survival. The NF-κB activated pathways include activation of Akt, the cell-survival kinase, which promote the survival of damaged cells, leading to tumor transformation (Figure 2).

**Curcumin: Advantages of Early Induction of Apoptosis**

Curcumin (diferuloylmethane), the active ingredient from the spice, turmeric, has been recognized for its anti-oxidant

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**Figure 2:** DNA damage response (DDR) pathways (see text).
and anti-inflammatory properties, and more recently for its ability to block phosphorylation events through its inhibition of phosphorylase kinase [28-31]. Protein kinases catalyze the transfer of high energy phosphate bonds from ATP to either serine/threonine or tyrosine residues [32], but usually not to both. This is because protein kinases usually allow only one configuration of its substrate binding site [32]. However, this is not the case with phosphorylase kinase, which is a protein kinase with multiple specificities attributable to its unique structure [33,34]. Phosphorylase kinase is a unique enzyme in which the spatial arrangements of its specificity determinants can be manipulated so that this enzyme can transfer high energy phosphate bonds to substrates of different specificities, including serine/threonine, tyrosine and even inositol. This is achieved by the presence of a hinge joint, which allows the manipulation of the size of the substrate binding site, and the ability to change the shape of the substrate binding site in two planes by either binding to Mn or Mg [34]. Curcumin is a well-known selective and non-competitive phosphorylase kinase inhibitor [35]. Its ability to inhibit the phosphorylation of a wide number of molecules with different substrate specificities is dependent on its ability to inhibit phosphorylase kinase [28-31].

Curcumin is hydrophobic and is poorly absorbed when taken orally, with negligible blood levels of the conjugated molecule even with high doses. However, the topical preparation appears to be effective on damaged skin [28-31]. The mechanism for the apparent rapidity of the observed repair in burns and acute skin damage is thought to be due to early curcumin-induced apoptosis, which allows new healthy (undamaged) cells to be formed, associated with rescue from cell-cycle arrest, which allows for rapid repair.

The damaged cell is programmed to always attempt to repair the DNA damage even if the healing process is prolonged, and even if the results are less than optimal, such as imperfect repair or excessive scarring. It is only when multiple attempts fail, that the cells undergoes “replication stress-induced apoptosis”, which is achieved through JNK-dependent pathways. The salutary effects of curcumin gel on thermal and solar burns may be accomplished through curcumin-induced apoptosis, which has the advantage of the following:

(1) Early apoptosis, perhaps achieved by by-passing the DDR pathway through inhibition of ATM kinase, a phosphatidylinositol-3K-like kinase which is involved in sensing DNA damage and initiating the DDR response.

(2) Selective apoptosis of DNA damaged cells through selective cellular expression of phosphatidylinositol 3-kinase family. Since all three phosphatidylinositol-3 kinase-like kinases (ATM, ATR and DNA-PK) are induced by the presence of double stranded breaks (DSBs) in the DNA, only the damaged cells with DSBs secrete the inositol-containing kinases. These kinases are activated by phosphorylation, perhaps achieved though phosphorylase kinase and inhibited by curcumin (phosphorylase kinase inhibitor). The selectivity observed with curcumin in DNA damaged cells is achieved by selective production of these inositol-containing kinases in damaged cells with DSBs. This selectivity ensures that non-damaged cells are not killed by apoptosis. The removal of the damaged cells allows the space necessary for the replacement of the damaged cells by new healthy cells.

(3) Rapid repair and rescue the DNA damaged cells from cell cycle arrest may be achieved by bypassing the DDR pathway, including cell cycle arrest. This may be achieved blocking the activities of ATR kinase, a phosphatidylinositol-3-kinase-like kinase in the DDR pathway. This allows for rapid repair of burns and acutely injured skin.

(4) Early removal of cells with DNA damage has the advantage of preventing the survival of potentially malignant cells with damaged DNA. This is particularly significant for repair of severe burns, including severe solar and radiation burns from ionizing radiation. Apoptosis with curcumin has also been reported to be achieved through other pathways, including depletion of Akt by blocking NF-kB activation.
[36,37]. It is possible that the action of curcumin through these additional pathways may synergize and amplify its apoptotic activity.

The rapidity of the healing observed after severe burns induced by curcumin gel (Figure 3a and Figure 3b) is thought to illustrate the early decision-making in favor of apoptosis, as well as by successful rescue from cell cycle arrest. Complete resolution without residual scarring suggests early replacement of the damaged cells by new healthy cells in burned skin treated with curcumin gel (Figure 3c and Figure 3d). The same rapidity of healing is also seen with solar burns (Figure 4a and Figure 4b), in which blister formation is aborted within 2 days of treatment with curcumin gel (Figure 4b). The healing of precancerous lesions (actinic keratoses) seen in Figures 5 and Figure 6 may also be achieved by killing and removal of the precancerous cells by curcumin-induced apoptosis.

It is hypothesized that the salutary effects of curcumin gel may be achieved by inhibiting phosphatidylinositol 3-kinase-like kinases (ATM, ATR and DNA-PK) in the DDR pathway through inhibition of phosphorylase kinase (summarized in Figure 7). Rapid healing and reversal of burns and solar radiation burns and removal of precancerous lesions may prevent further malignant transformation in photo-damaged skin.

The strategy of using an extraneous drug such as curcumin to selectively choose apoptosis versus repair in severely damaged cells, frees the cells from the decision-making process of whether or not to die. The concomitant
ged cells by new uninjured cells, thus resulting in seamless and painless tissue regeneration.

**Summary**

We have detailed the DDR pathway triggered by DNA damage, which leads to cell cycle arrest, nucleotide excision and repair, and nucleotide replication. This is a laborious process resulting in recruitment of multiple proteins to the histones for nucleotide replication and repair. Mistakes are likely to be made if large segments of DNA are damaged or if both strands are damaged, leading to formation of premalignant and malignant cells. In addition, the need to channel all available resources to the repair processes, result in cell cycle arrest and cessation of healing.

Our clinical patients showing rapid healing with curcumin gel demonstrates the uniqueness of curcumin-induced...
Pathways is also thought to be achieved through inhibition of phosphorylase kinase [1].

Phosphorylase kinase is a unique kinase in which the spatial arrangements of the specificity determinants can be manipulated to allow phosphorylase kinase to transfer high energy phosphate bonds from ATP to substrates of different specificities [32-34]. We have previously reported that phosphorylase kinase is capable of phosphorylating both serine kinases and tyrosine kinases in the NF-kB-dependent MAP kinase pathways. A previously unreported finding is that phosphorylase kinase may also be able to transfer high energy phosphate bonds to protein kinases of the phosphatidylinositol 3-kinase families including ATM (ataxia telangiectasia mutated) kinase, ATR (ataxia telangiectasia rad 3 related) kinase and DNA-PK (DNA-protein kinase). We propose that phosphorylase kinase may have an important role in the DDR (DNA damage response) pathways by activating the early response enzymes, such as ATM, ATR, and DNA-PK. This phosphorylation step has been previously attributed to autophosphorylation by other investigators. However, the efficacy of curcumin, a known phosphorylase kinase inhibitor, in blocking the DDR pathways is also thought to be achieved through inhibition of phosphorylase kinase [1].

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Figure 7: Curcumin-induced apoptosis and rescue from cell cycle arrest: Removal of damaged cells by curcumin-induced apoptosis allows the space for regeneration by new healthy cells. Rescue by curcumin from cell cycle arrest allows for rapid healing.
pathway suggests the possibility that phosphorylase kinase may also be involved in activation of phosphorylation events in this pathway (Figure 7). The previously reported induction of apoptosis by curcumin through NF-κB [36] and Akt [37] dependent pathways may also be achieved through inhibition phosphorylase kinase [28-30]. These additional pathways targeted by curcumin through phosphorylase kinase inhibition may synergize to enhance its apoptotic activity.

In conclusion, it is likely that the rapid healing achieved in burns and sun-burns may be achieved by two factors. First, the capability of curcumin to block phosphorylase kinase-dependent phosphorylation through NF-κB-de- pending pathways allows the damaged cells to undergo rapid apoptosis using alternative pathways other than the usual DDR pathways. Second, the capability of curcumin to inhibit the phosphatidylinositol 3-kinase family also allows the damaged cell to undergo early apoptosis rather than be involved in a prolonged, unsatisfactory repair process, with increased risk of tumor transformation. This early decision-making achieves early induction of apoptosis in damaged cells, providing the space necessary for replacement by new healthy cells. The damaged tissues, “rescued” from “cell cycle arrest” achieves rapid healing without residual scarring as shown our patients with solar and thermal injury treated with topical curcumin.

References


