Mini review

CYTO-BIOLOGICAL ACTIVITIES OF LACTAPTIN: AN INSIGHT INTO DESIGNING OF NEW CANCER THERAPAUTICS

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ABSTRACT

Background and objectives: Designing of effective anticancer-based peptides has been challenging these days probably due to the instability of the peptides in serum as well as low sensitivity and resistance of cancer cells to these peptides.
Methodology: published papers addressing anticancer activity of lactaptin has been reviewed extensively.
Results: Lactaptin, a peptide from proteolytic cleave of kappa casein human milk has been shown to play a number of roles including Program cell death, Genetic material fragmentation, suppression of metastasis, cytotoxicity to the cancer cells as well as caspases activation.
Conclusions: Lactaptin shows promising activity against cancer cells. Accordingly, enhancing lactaptin activity will greatly enhance its therapeutic efficacy and will provide insight into designing of new therapeutics.

Keywords: Lactaptin, Apoptosis, DNA fragmentation, Cytotoxicity, Caspases

1. INTRODUCTION

Designing of an effective anticancer molecule remains one of the challenges of this century probably due to insensitivity of conventional chemotherapeutics or resistance by the cancer cells [1]. Anticancer molecules that are stable in the serum and are very specific to cancer cells in term of toxicity are very necessary to improve the efficacy of the existing molecules and developed new candidates. Lactaptin, a candidate generated from proteolytic Cleave of human milk kappa-casein has shown a promising tumoricidal activity against variety of cancer cells [2,3]. First isolated, purified and characterized by Nekipelaya and colleagues 10 years ago [2], it is made up of 74 amino acids with molecular weight of 8.6 kDa [2]. Several analogs of this peptide have been designed, out of which only RL2 with amino acids sequences similar to that of lactaptin was able to induce cancer cell death invitro in both human and mice without affecting normal cells. This recombinant peptide refers to as lactaptin analog (RL2), have it tumoricidal activity tested on cultured human cell [4,5]. This peptide demonstrated tumoricidal activity against cancer metastasis of both human and mice cancer cells and also induces apoptosis in the both cells [6,7]. The safety of this peptide has also been investigated, the pharmacokinetics as well as the toxicity of the peptide has been studied [8]. It was shown that lactaptin is safely distributed and biodistribution reduces the concentration available to cancer cell [8, 9]. This bioactive peptide, from human milk has many functions including cancer cell lysis, apoptosis, suppression of metastasis, activation of caspases among many others. The purposes of this paper are to discuss the tumoricidal activity of Lactaptin, mechanisms of actions, prospects and challenges.

2. MECHANISM OF LACTAPTIN INDUCE PROGRAM CELL DEATH

The most noted biochemical events of lactaptin induced program cell death were activation of caspases, changes of cytoplasmic membrane and mitochondrial membrane dissipation. It was also

found that program cell death was accompanied by released of phosphatidyl serine in the plasma membrane. Koval et.al. reported that the recombinant analogue of lactaptin downregulates Bcl-2 expression and induces program cell death independent of p53 [6,7,11]. Presently, identified pathways of the cell entrance of peptides and proteins into cells include endocytosis facilitated by specific cell structures and direct entrance across the cell membrane made possible by properties of these peptides. It has been demonstrated that RL2 gains entry into human neoplastic and non-neoplastic cells through binding onto skeletal cell structures. Molecular studies also indicated that Lactaptin analog cross the cell membrane partly through lipid raft mediated dynamin-independent pinocytosis and partly through direct penetration across the plasma cell membrane. The activation of the caspases by the peptide [11] might be probably due to the production of reactive oxygen species. Another study of mechanism of action of different peptides has linked the activation of the caspases to production of ROS followed by release of cytochrome C. Although the extrinsic pathway has not been reported as mechanism of action of this peptide, the pathway might also be possible due to receptors of peptides preset at cell membrane. The extrinsic pathway of cell death might start with reception of signals from the peptide onto the death receptor (FADD), the peptide in this case serves as a death receptor ligand equivalent in function to the CD95L and TNFα respectively. Activation of the death receptor lead to the instigation of Pro-caspases 8 and 9 to caspases 8 and 9 (initiation caspases) and activation of caspase 7 which ultimately leads to apoptosis.

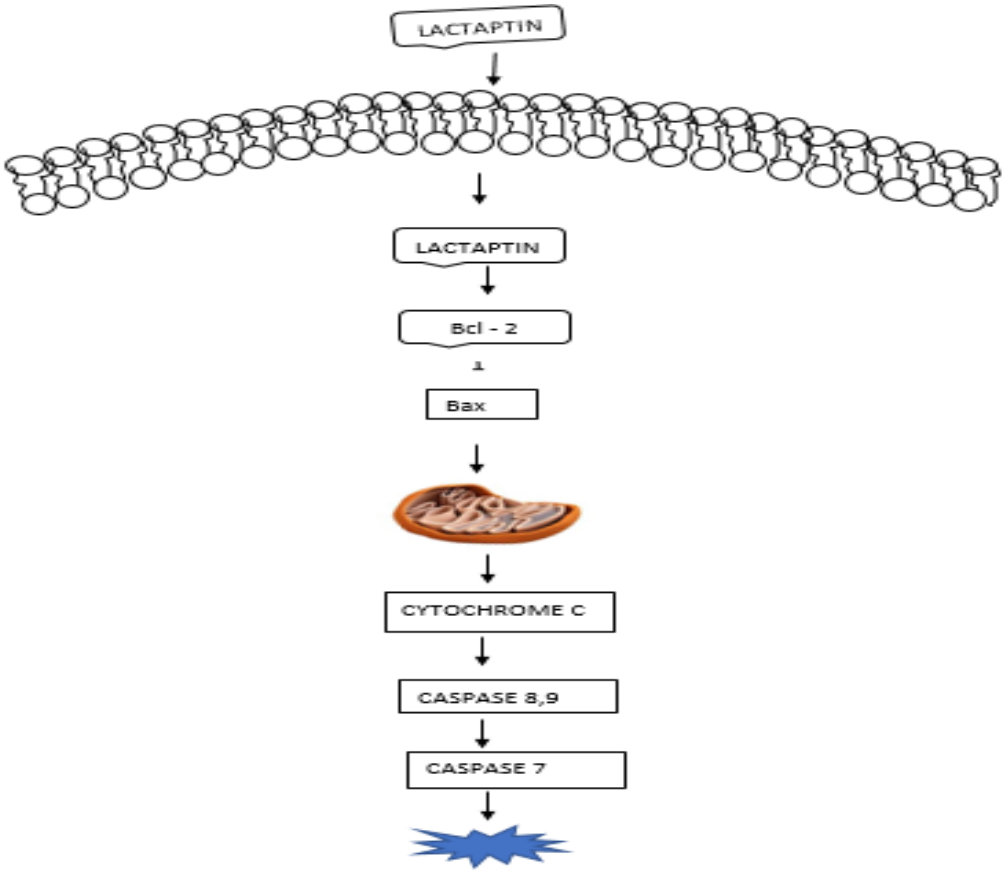

Figure 1: Lactaptin induced apoptotic pathway

3. DNA FRAGMENTATION

DNA fragmentation involved the series of event which leads to the damage of genomic DNA. This phenomenon often occurs following treatment of cancer cells with anti-cancer peptides capable of causing program cell death. Lactaptin analog penetrates the cell through the membrane into the cytoplasm [9] favoring cellular cascades initiating this damage. Although some are seen DNA fragmentation as not completely necessary for program cell death [12, 13], genomic DNA

fragmentation differentiated dead cell from a living cell and facilitated dead cell uptake by phagocytosis as well as improving the whole process of apoptosis [13-16]. Particular enzymes have been pinpointed in cleavage to the genomic materials, out of which Caspase Activated DNase (CAD) located in nucleus has been in the forefront [17-19]. CAD consist of essentially proteolytic enzyme with nuclease activity that break genomic DNA in the course of apoptosis and enhances cell diffentiation. They exert their activity through dimerization of it monomers which induces the formation of sharp molecular scissor-like structure that cut double stranded DNA [13]. An important mechanism of inhibition of the activity of this nuclease might be prevention of the formation of this dimer, formation of dimer from CAD and ICAD (inhibitor of CAD) [13] The initial mechanism of CAD activity starts with inactivation of ICAD by proteolytic cleavage and irreversible inactivation of DNA repair mechanism both carried out by caspase 3 which is one of the execution caspases [13]. Lactaptin has been shown to activates caspase 7 [11], which may play the same role as caspase 3, thus, lactaptin may causes cleavage of ICAD through activation of caspase 7 and inactivate DNA repair mechanism which can ultimately leads to DNA fragmentation.

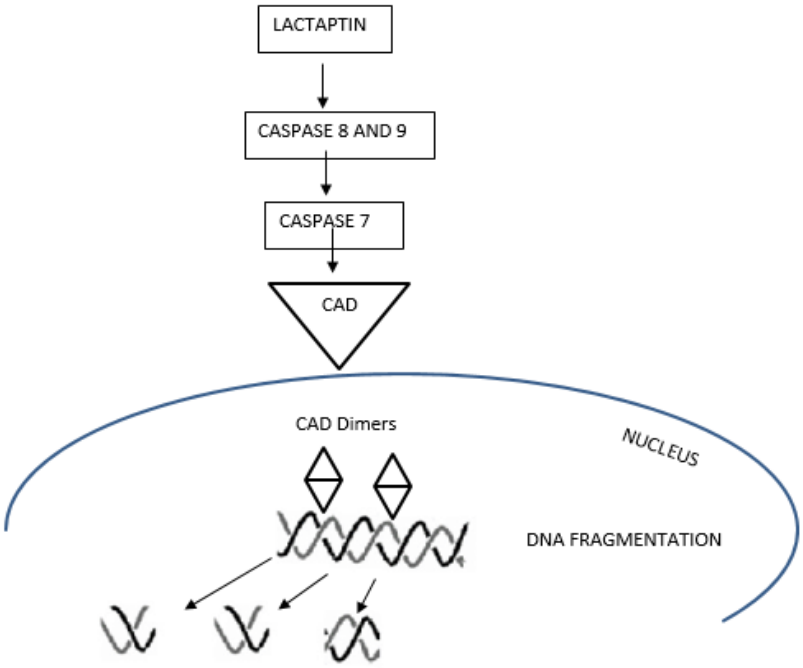

Fig.2. DNA Fragmentation by CAD Dimers

4. SUPPRESSION OF METASTASIS

Metastasis depict many-stage cyto-biological process involving cascades in which transformed cells travels to distance tissues and adapted to the new microenvironment. In metastasis, cancer cell Invade surrounding tissues and blood vessels, travel through the blood stream to reinvade nearby tissues. The transformation of cell' both epigenetic and genetic material has been one of the factors driven this cascade. Lactaptin as an anticancer peptide have been shown to act against varieties of cancer cells and has been shown to inhibit the metastasis of both mice and human cell triggering them to apoptosis [6,7]. Quantitative measurement of metastatic tissues shows three times decrease in hepatic metastasis of a mice administer with recombinant lactaptin analog compare with control mice [6,7]. In the same vein, recurrent administration of RL2 significantly extended the life of experimental animal injected intravenously with cancer cells [7]. The peptide essentially delayed tumor in experimental animals against their control counterpart [7]. Inhibition of tumor Metastatic rate of RL2 has been calculated as 43% [7] which indicate the antimetastatic activities of the peptide and the need to modify it and improve it therapeutic applications.

One good attribute of anti-metastatic candidate is that it must be able to block the proliferation and persistence of cancer cells travelling into distant tissues and not merely stop seepage of individual

cells from the primary tumors [20]. Therefore, designing of new cancer therapeutics should consider this fact as many anticancer therapeutic fail to achieve this. Lactaptin should therefore be optimized to improve it therapeutic efficacy and serum stability. As noted by Gilbert and Hemann [20, 21], it is likely that vicinity of metastasis site formed resistance against anticancer therapeutics, thus become chemoprotective. It is also stated by Aguirre-Ghiso [20, 22] that agents that are toxic to cell inhibiting division cycle and active growth may be resisted by slow growing micro metastasis, in this regard, improved therapeutics are urgently needed.

Many of the biochemical event leading to the metastatic cascade can be controlled with number of intervention such as small molecules, monoclonal antibodies and miRNA [23]. Due it inhibition of metastases, lactaptin can play critical role in regulating the spread of these cancer cells by targeting this cascade (Fig. 3). The major event in metastasis cascade include primary tumor formation, local invasion, survival of the tumor in circulation, distant organ arrest, extravasation, micro metastasis formation, metastasis colonization and finally clinical detected metastasis [20] (fig 3). Lactaptin may emerge as one of the promising therapeutics that can target the whole event of metastasis cascades. In this way, our capacity to successfully treat malignancy is to a great extent subject to our ability to comprehend—and maybe even turn around—the metastasis cascades.

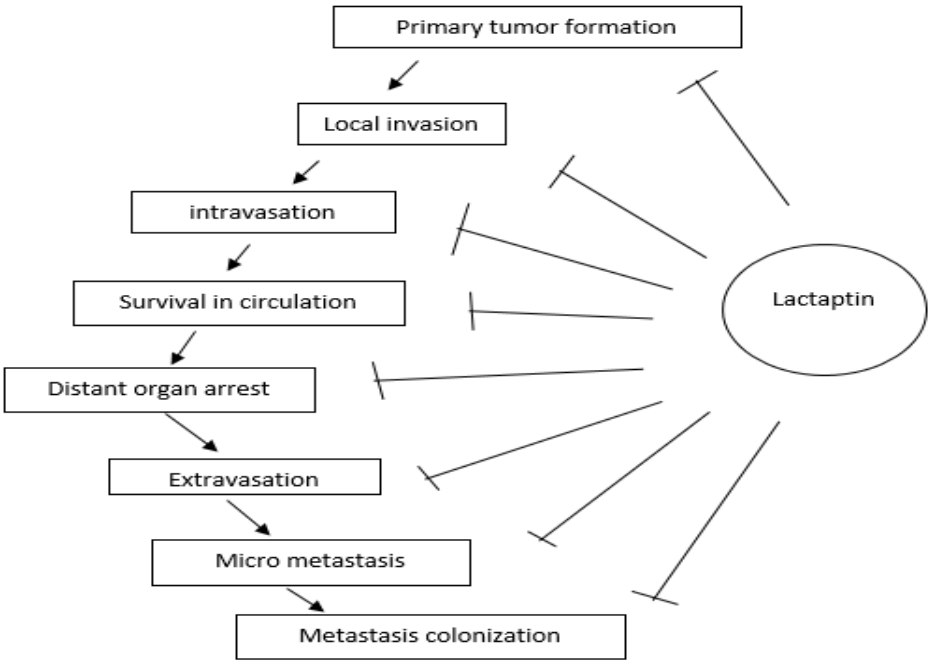

Fig. 3. Lactaptin inhibition of Metastasis cascades

5. CYTOTOXIC ACTIVITY OF LACTAPTIN

MTT has been exploited to check the cytotoxic activity of lactaptin. The cytotoxic activity of RL2 was analyzed using human endometrial cancer cells in which cultures of both neoplastic and non-neoplastic human endometrial [6] were carried out by digestion of endometrial tissue obtained from surgery material. Results indicated that RL2 employ it cytotoxic activities to the human endometrial cancer with apoptosis – like features [6]

6. ENHANCING OF LACTAPTIN WITH TUMOR SPECIFIC PEPTIDE

Enhancement of lactaptin with tumor specific peptide may play important role in helping lactaptin exerting it therapeutic effects. poor penetration of cancer peptides might be the limitation of some anticancer agent for exerting their activity. Systemic administration with iRGD, a tumor-targeting and -penetrating peptide, enhanced the therapeutic efficacy of drugs of various compositions, including a monoclonal antibody (trastuzumab), a small molecule (doxorubicin) and nanoparticles (nab-paclitaxel and doxorubicin liposomes). Thus, co-administration of iRGD may be an important way to improve the usefulness of anticancer drugs while decreasing their side effects, a main target of cancer treatment investigations [25,26]. An in vitro study of recombinant lactaptin with a number of fusion proteins has been carried out against two different cancer cell lines MCF-7 and MDA-MB-231 [25,26]. The result indicates that fusion proteins enhanced with lactaptin exert inhibition of the cell lines proliferation and antitumor activity of the enhanced peptide is greater than the lactaptin alone [25,26]. In vivo studies of one of the fusion proteins with lactaptin, T3 – RL3 in mouse xenograft using cancer cell lines indicates that T3 – RL3 inhibit tumor higher than that of the RL2 in comparison [25,26]. Accordingly, the enhancement of lactaptin with tumor specific proteins will greatly increase it specificity and therefore increase it overall therapeutic effects. A number of possible modifications of peptides has been provided [27]. This include adjustment of C- and N-terminal of peptide, hybridization, cyclization and substitution of amino acids in the existing peptide [27]. Accordingly, enhancing lactaptin activity will greatly enhance it therapeutic efficacy and will provide insight into designing of new therapeutics.

7. Discussion

Effective drug development of anti-cancer molecules is desperately required considering the very fact that resistance rises on most the chemotherapeutic agents used at the moments. Peptides has shown promising role in resolving variety of ailments, their role in cancer medicine and on the far side has been well acknowledged [26-29]. Many properties this peptide such as affordability, promptly tissue penetration, low immunogenic and easy manipulation has made it suitable for research purposes and readily available to be developed into antitumor drug. Yavari et. al. [30] has review the use of peptides as therapeutic agents both alone and in conjunction with other peptides [30], indicating the beneficial use of peptide for follow up cancer treatment as well as for diagnostic process. There has been extensive research on milk peptides often relating these peptides to many functions including anti-cancer, anti-metastasis, anti-microbial, antioxidant effects and immunomodulatory properties [27]. Peptide from milk are generally tolerated with well bioavailability, a property that will enhance successful treatment of cancer ailments [27].

As at the moment, research and development of cancer therapeutics based on peptides obtained from milk capable of causing apoptosis, cytotoxicity and anti-metastasis has become very popular and many more are in progress [27, 28]. Lactaptin, a pepetide with a potent property to induced program cell death of many different cancers activate proteases- the caspases that execute cancer cell to apoptosis [6,7]. An analog of this particular peptides possesses cytotoxic activity to human and mice cancer cell both in vivo and invitro. The pre-clinical trial studies have been well established following the evaluation of its anti-tumor activities in breast cancer cell mouse xenograft [8,9]. The safety of this peptide has also been confirmed by toxicity and pharmacokinetics studies [8].

One of the major downsides was that laptaptin was distributed evenly through the body and therefore reduces the amount available to cancer cells. Improvement of the anti-cancer efficacy of RL2 through conjugation with tumor specific peptides is expected to promote delivery of RL2 to target tissues [9]. Tumor- specific peptides proved as a better target for delivering therapeutic substances and are used for a number of purposes including delivering vehicles to pro-apoptotic peptides, genes, therapeutic drugs and cytokines [9]. A number approaches can be used to increase the half-life of this peptide as well as it size, for example conjugation with polyethylene glycol (PEG) can be performed [31]. However, the barrier to this approached is production of antibodies against the glycation product. A new method has been devised to take care of this problem which involve conjugation with 864 amino acid peptides referred to as XTEN which also increases peptides half-life [31].

8. Conclusions

Review of lactaptin as a new candidate molecule for antineoplastic agent has been discussed. The peptide has been found to play vital role in program death, cytotoxic to cancer cells, activation of caspases and suppression of metastasis. In general, the peptide shows promising activity against cancer cells. consequently, enhancing it activity can greatly enhance it therapeutic effectiveness and can give insight into coming up with of latest cancer therapeutics.

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