Mini review

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

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 review
Results: Lactaptin, a peptide from proteolytic cleave of kappa casein human milk has been show 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 it 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 in this century probably due to insensitivity of conventional chemotherapeutic or resistance by the cancer cells [1] Anticancer molecule that are stable in the serum and are very specific to cancer cell in term of toxicity are very necessary to improve the efficacy of the existing molecule 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]. A recombinant of this peptide lactaptin, refer to as lactaptin analog (L2) has previously been synthesized and it tumoricidal activity tested on cultured human cell [4,5]. The recombinant has been demonstrated to show tumoricidal activity against cancer metastasis of both human mice cancer cells and also induces apoptosis in the both cells [6,7]. The safety of this peptide has been investigated, the pharmacokinetics as well as the toxicity of the peptide has been carried out [8]. The research shows that lactaptin is safely distributed and biodistribution reduces the concentration available to cancer cell [8, 9]. Bioactive peptide, lactaptin 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

Apoptosis also called program cell death involves removal of unwanted cells by an organism during early stages of development, development of organs, response to viral infected cells as well as some disease state such as cancer. It is normally carried out to maintain homeostasis. The identifiable features of this pathway include blebbing of the membrane, occurrence of greatly condense chromatin, endonucleolytic process activation leading cleavage of the DNA of the chromosome (10). Techniques in Molecular Biology such as Flowcytometry and western blotting have been used to exploit the mechanisms by which lactaptin induces apoptosis to cancer cell death [11]. Activation of

the caspases that initiate and execute apoptosis has been reported [11] The recombinant lactaptin dissipate mitochondrial membrane potential thereby activation of the caspases that initiate apoptosis following the administration of this peptide has specially been noted i.e. caspases 8,9 and execution caspase 7[6,7,11]. Phosphotidyl serine release on the surface of the plasma membrane accompanied induction of apoptosis by the lactaptin recombinant analogue. Research has shown that the recombinant analogue of lactaptin downregulates Bcl-2 expression and induces p53-independent cell death [11]. Lactaptin causes cell membrane raptured thereby permitting the peptide (lactaptin) into the cell interior (Fig 1). The peptide initiated intrinsic pathway of cell death by generation of Reactive Oxygen Species (ROS). The ROS enter into endoplasmic reticulum which causes the release of calcium ion. The calcium ion in turn causes the depolarization of mitochondria which subsequently lead to release of cytochrome C from the mitochondria (Fig 1). The release of cytochrome C causes the activation of initiation caspases and subsequently execution Caspases as reported [11]. The extrinsic pathway of cell death starts 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 – CASPASS 8 and 9 to caspases 8 and 9 (initiation caspases) and activation of caspase 7 which ultimately leads to apoptosis (Fig. 1)

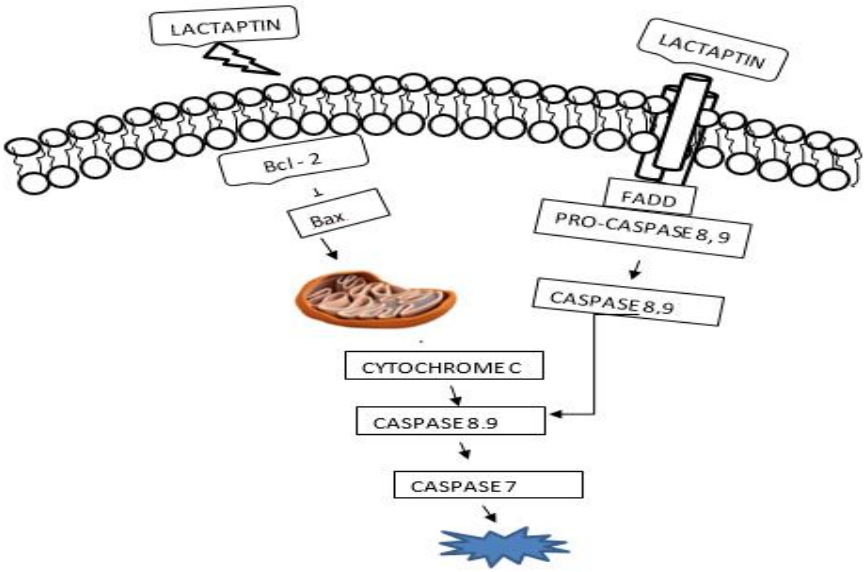

Figure 1: Lactaptin induced apoptotic pathway

3. DNA FRAGMENTATION

DNA fragmentation involved the series of event which leads to the damage of genomic DNA. DNA is often fragmented in the case of treatment of cancer cells with anti-cancer peptides capable of causing program cell death and subsequently the damage of the genetic material. Lactaptin penetrates the cell through the membrane and enters into the cytoplasm. Release of ROS to the endoplasmic reticulum cause the release of Ca2+ which depolarizes membrane potential of mitochondria and concomitant of cytochrome C release (fig 1). The licking of the cytochrome marks the beginning program cell death event. Cellular DNA fragmentation occurs as a result of apoptosis and marks the end of cell's life. Although some are seen it as not completely necessary [12, 13], genomic DNA fragmentation differentiated dead cell from live 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. The CAD exert it activity through dimerization of it monomers which induces the formation of sharp molecular scissor-

like structure that cut double stranded DNA. 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.

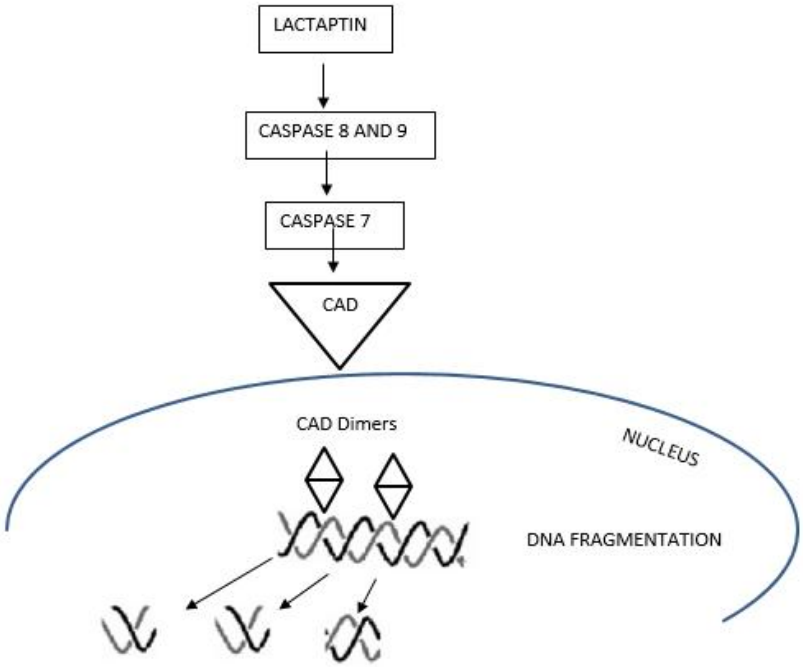

Fig.2. DNA Fragmentation by CADD Dimers

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. In the same vein, recurrent administration of RL2 significantly extended the life of experimental animal injected intravenously with cancer cells. 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 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.

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.

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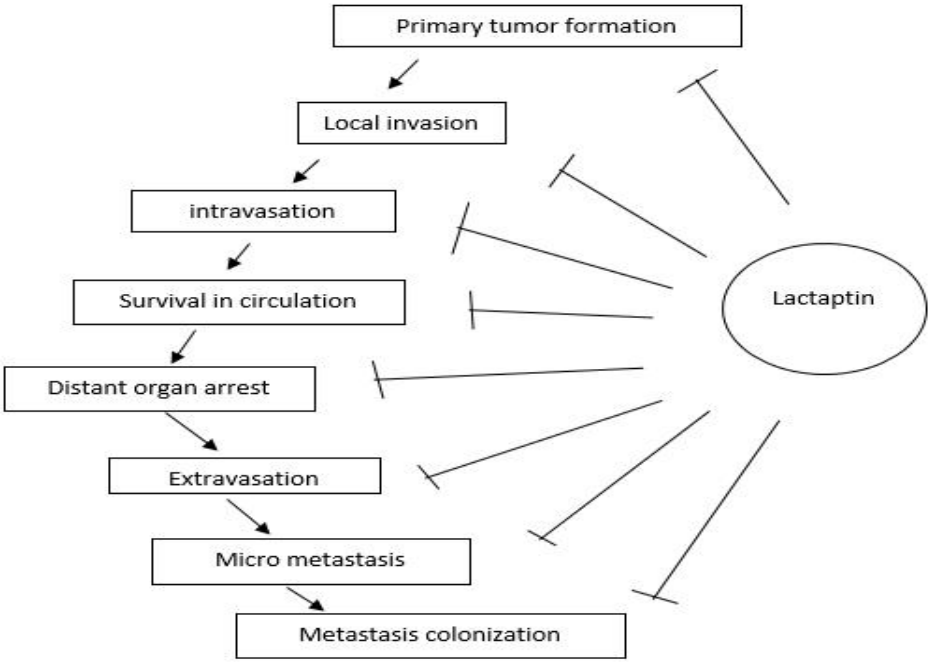

Fig. 3. Lactaptin inhibition of Metastasis cascade

5. CYTOTOXIC ACTIVITY OF LACTAPTIN

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test is commonly reduction assay used to analyzed the viability of cells against compound thought to possess cytotoxic activity [24]. The method is widely employed in molecular biosciences and related disciplines and has become prominent in research laboratories and published literatures [24]. The basic principle behind this method involve the conversion of MTT to formazan by metabolically active living cells indicated by color formation while dead cell could not [24]. MTT has been exploited to test the cytotoxic activity of lactaptin. The cytotoxic activity RL2 was analyzed using human uterine cancer cells in which cultures of both normal and malignant human uterine [6] were carried out by digestion of endometrial tissue obtained from biopsy material. Results indicated that RL2 employ it cytotoxic activities by hormone dependent passion of the human uterine cancer with apoptosis – like features [6]

6. ENHANCING OF LACTAPTIN WITH TUMOR SPECIFIC PEPTIDE

Enchantment of lactaptin with tumor specific peptide may paly important role in helping lactaptin exerting it therapeutic effects. poor penetration of cancer peptides might be he limitation of some anticancer agent for exerting their activity. Systemic administration with iRGD 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 a treasured 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.

References

1. Lozza C, Navarro-Teulon I, Pelegrin A, Pouget J-P, Vives E. Peptides in receptor-mediated radiotherapy: from design to the clinical application in cancers. Front Oncol. 2013;3(247):1–13.

2. Nekipelaya VV, Semenov DV, Potapenko MO, Kuligina EV, Kit Yu, Romanova IV and Richter VA. Lactaptin is a human milk protein inducing apoptosis of MCF-7 adenocarcinoma cells. Dokl Biochem Biophys. 2008; 419: 58-61.

3. Vlassov VV, Richter VA, Semenov DV, Nekipelaya VV, Kuligina EV and Potapenko MO. Peptide inducing apoptotic death of human cancer cells. 2008; Patent RF N 2317304.

4. Tikunova NV, Semenov DV, Babkina IN, Kuligina EV, Koval O A., Fomin A S, Matveeva V A, Matveev L E, Matveev AL, Richter VA. Recombinant plasmid DNA pFK2, providing synthesis of the recombinant peptide which is the analog of human kappa-casein, and recombinant peptide – the analog of human kappa-casein fragment, with the apoptotic activity against human tumor cells. 2010; Patent RF N.2401307.

5. Semenov DV, Fomin AS, Kuligina EV, Koval OA, Matveeva VA, Babkina IN, Tikunova NV and Richter VA. Recombinant analogs of a novel milk pro-apoptotic peptide, lactaptin, and their effect on cultured human cells. Protein J. 2010; 29: 174-180.

6. Koval OA, Fomin AS, Kaledin VI, Semenov DV, Potapenko MO, Kuligina EV, Nikolin VP, Nikitenko EV and Richter VA. A novel pro-apoptotic effector lactaptin inhibits tumor growth in mice models. Biochimie. 2012; 94: 2467-2474.

7. Koval OA, Tkachenko AV, Fomin AS, Semenov DV, Nushtaeva AA, Kuligina EV, Zavjalov EL and Richter VA. Lactaptin induces p53-independent cell death associated with features of apoptosis and autophagy and delays growth of breast cancer cells in mouse xenografts. PLoS One. 2014; 9(4): e93921

8. Bondarenko DA, Richter VA, Kuligina EV, Koval OA, Fomin AS, *et al*: Toxicity studies and pharmacokinetics of Lactaptin. Russian Journal of Biopharmaceuticals 2015; 7: 40-47.

9. Nemudraya AA, Kuligina EV, Ilyichev AA, Fomin AS, Stepanov GA, Savelyeva AV, Koval OA, Richter VA. Selection of antitumor displayed peptides for the specific delivery of the anticancer drug lactaptin. Oncol Lett. 2016; 12(6):4547–4555.

10. Wu M, Ding H, Fisher DE. Apoptosis: Molecular Mechanisms. Encyclopedia of Life Sciences. Nature Publishing Group; 2001.

11. Richter VA, Vaskova AA, Koval OA, Kuligina EV. Antitumor Potential of Lactaptin. Biol Med (Aligarh). 2015; S2:004.

12. Samejima K, Earnshaw WC. Trashing the genome: the role of nucleases during apoptosis. Nat Rev Mol Cell Biol. 2005; 6:677–688.

13. Larsen BD, Sørensen CS. The caspase-activated DNase: apoptosis and beyond. The FEBS Journal. 2017; 284 :1160–1170

14. Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P Et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature. 2009; 461:282–286.

15. Radic M, Marion T, Monestier M. Nucleosomes are exposed at the cell surface in apoptosis. J Immunol. 2004; 172: 6692–6700.

16. Yan B, Wang H, Wang H, Zhuo D, Li F, Kon T, Dewhirst M, Li CY. Apoptotic DNA fragmentation factor maintains chromosome stability in a P53-independent manner. Oncogene. 2006; 25:5370–5376.

17. Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell.1997; 89:175–184.

18. Enari M, Sakahira H, Yokoy ama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature.1998; 391: 43–50.

19. Halenbeck R, MacDonald H, Roulston A, Chen TT, Conroy L, Williams LT. CPAN, a human nuclease regulated by the caspase-sensitive inhibitor DFF45. Curr. Biol. 1998; 8:537–540.

20. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011; 147:275–292.

21. 21 Gilbert LA, Hemann MT. DNA damage-mediated induction of a chemo resistant niche. Cell. 2010; 143:355–366.

22. Aguirre-Ghiso, J A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. Nat. Rev. Cancer. 2007; 7: 834–846.

23. Guan X. Cancer metastases: challenges and opportunities. *Acta Pharmaceutica Sinica B*. 2015; 5: 402–418.

24. Riss TL, Moravec RA, Niles AL, Benink HA, Worzella TJ, Minor L. Cell viability assays. In: Sittampalam GS G-EN, Arkin M, et al., editors. Assay Guidance Manual. Bethesda (MD), USA: Eli Lilly & Co and National Centre for Advancing Translational Sciences; 2012.

25. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, et al. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. Science. 2010;328(5981): 1031–5.

26. Nemudraya AA, Makartsova AA, Fomin AS, Nushtaeva AA, Koval OA, Richter VA, et al. Tumor-Specific Peptide, selected from a Phage Peptide Library, Enhances Antitumor Activity of Lactaptin. PLoS ONE. 2016; 11(8): e0160980.

27. Hu C, Chen X, Zhao W, Chen Y, Huang Y. Design and Modification of Anticancer Peptides. Drug Des.2016; 5:138.