# INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
## SHORT ABSTRACT OF THESIS


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Programme of Study : **Ph.D.**

Thesis Title: **Functional Characterizations of Plant Uracil Phosphoribosyltransferase (UPRT) and Phytaspase for their Potential in Cancer Therapy**


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Thesis Submitted to the Department/ ~~Center~~ : **Department of Biosciences and Bioengineering**



Date of completion of Thesis Viva-Voce Exam : **12-04-2014**

Key words for description of Thesis Work : **AtUPRT, Phytaspase, Caspase-like protease, Cancer therapy**


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## SHORT ABSTRACT


In burgeoning field of cancer research, plant bioactive compounds and plant therapeutic proteins have been found very effective on persisting drug resistant cancer. Although many plant derived compounds are either in market or under clinical trials, there is still need of in-depth analysis on the plant functional proteins. Uracil phosphoribosyltransferase (UPRT) and phytaspase caspase-like protease are two such functional plant proteins, which are reported to have their functional counterparts in bacterial (bUPRT) and mammalian (caspase) system, respectively. In turn, these counterparts have been already very well exploited for their major role in the cancer treatment. Bacterial UPRT, pyrimidine salvage pathway enzyme converts the prodrug 5- FU into toxic metabolite 5-FUMP inside the cancer cells leading to apoptosis. Whereas, caspases are intracellular mammalian cysteine dependent proteases, which are mainly involved in the execution of apoptosis. However, thorough characterization of recombinant AtUPRT and phytaspase, and their potential in complementing their functional counterparts in cancer treatment have been conducted in detail in this current thesis. More specifically, this thesis addresses the raising question of how far these two proteins mimic their counterparts in contributing themselves in the cancer therapy either by gene or by protein therapeutics. In **Chapter 1**, the emerging importance of cancer gene and protein therapy has been discussed. It also brings the deeper value of preliminarily investigated or yet to be explored plant suicide genes encoding therapeutic protein's application in cancer therapy into the limelight. More specifically, it deals exclusively with the significance of *Arabidopsis thaliana* uracil phosphoribosyltransferase (AtUPRT) and *Nicotiana tabacum* phytaspase. The chapter deciphers the possibilities of utilizing the potential of phytaspase in cancer therapy. The last section of this chapter encompasses on the future significance of devising these recombinant proteins into the mammalian system either exogenously or by expression and finally, through their mode of action sensitizing the cancer cells towards chemotherapeutic drugs. At the end, development of quantum dots embedded polymeric nanocarriers for delivering therapeutic agents have been illustrated.

**Chapter 2** began with the *in silico* investigation on *A. thialana* UPRT (AtUPRT), where AtUPRT was shown to have lower binding energy with its substrate 5-FU, as compared to *E. coli* UPRT. Cloning of AtUPRT into the mammalian expression vector has been delineated. Further, the therapeutic implication of AtUPRT expressed in HeLa cells by sensitizing the cells towards prodrug 5-FU was established by cell viability assay, cell cycle analysis and cell apoptosis analysis through flow cytometric analysis. The low survival efficiency of the AtUPRT expressing HeLa cells post 5-FU treatment was finally determined by quantitative clonogenic assay.

In **Chapter 3,** cloning (in bacterial and mammalian expression vector), expression and characterization of *Nicotiana tabacum* pre-prophytaspase and mature phytaspase in *E. coli* have been reported. This chapter is also stressed on the difficulties involved in purifying the bacterially expressed pre-prophytaspase and mature phytaspase. Recombinant mature phytaspase after cleaving GST tag retained its functionality towards caspase-8 substrate and its kinetic parameters were analyzed by enzymatic assay. Also theranostic potential of recombinant mature phytaspase (after removal of GST) bound to Mn doped ZnS QDs-Chitosan NPs (nanocomposites) have been elucidated. The quantum dots were useful for tracking and bio-imaging studies. The effect of phytaspasenanocomposites on HeLa cells in the presence of apoptosis inducing drug, cisplatin was analyzed using cell viability assay. Also the prominent chemo-sensitizing effect of transfected mature phytaspase in A549 (adenocarcinoma human alveolar basal epithelial) cells towards drug is reported in this chapter.

The final **Chapter 4** on conclusions and future prospects, highlights the emerging importance of this study as well as and the important pack of information obtained in the current thesis. In brief, for the first time, AtUPRT have been cloned in mammalian expression vector and expressed in HeLa cells. The sensitizing effect of its expression in cancer cells towards 5-FU was studied through various techniques. The other part of the section focused on phytaspase-caspase like protease. *N. tabacum* phytaspase was cloned and expressed in bacterial system. Its thorough sequence and structural characterization was carried out. Retaliation of functionality of recombinant phytaspase assessed by caspase activity assay illustrates its implication in cancer therapy. Thereafter, therapeutic efficacy of the recombinant phytaspase was established in the presence of cisplatin by its exogenous supply to the cancer cells upon loading onto quantum dots embedded polymeric nanocarriers. Similarly, phytaspase was also cloned in mammalian expression vector and transiently expressed to evaluate its anti-cancer potential on A549 cells in the presence of doxorubicin. The optimized protocols in this current work may serve as easier way to obtain these recombinant proteins avoiding the cumbersome process of isolating proteins from their native plant source. This detailed characterization information on these plant genes really serve as a platform for deeper investigation and holds their immense importance in the future of cancer therapy.