

# Cancer on the Cross-Wires

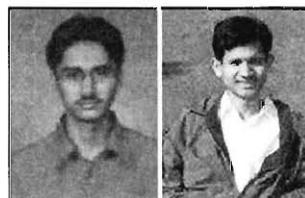
## A Possible Scheme for Cancer Cure

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Normal cells in our body that constitute a cellular society are not selfish and this is evident by the fact that they have evolved a phenomenal pathway whereby they cause their own death under conditions of stress that lie beyond the absorbing capacity of the cell. This phenomenon is known as 'Apoptosis' or 'Programmed Cell Death'. However, cancerous cells, which are indeed selfish and proliferate at the cost of their normal counterparts, evade the apoptotic pathway by hindering the mitochondrial release of the proapoptotic protein cytochrome c (Cyt c). Here, we have proposed a scheme whereby the power of proteasome (an intracellular protein chopper) could be harnessed to remove the obstruction placed by cancer cell to the apoptotic process and thereby pushing the cancerous cells to self-destruction, leading to a possible cure for cancer.

Cancer claims thousands of lives, both young and old every year. There are more than a hundred different kinds of cancer, but some are more common than others. Because cancer results from defects in fundamental cell regulatory mechanisms, it is a disease that ultimately has to be understood at the molecular and cellular levels. The common feature of all cancers is the unrestrained proliferation of cancer cells, which eventually spread throughout the body, invading normal tissues and organs and leading to death of the patient. Cancer cells typically display abnormalities in the mechanisms that regulate normal cell proliferation, differentiation and survival [1].

Surgery and immunotherapy are effective treatments for localized cancers but are unable to reach cancer cells that have spread to distant body sites. Treatment of these cancers therefore requires the use of chemotherapeutic drugs. Unfortunately, the



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Apoptosis is a process of programmed cell death whereby cells under stress undergo self destruction to arrest the propagation of their harmful effects to the rest of organism.

currently available chemotherapeutic agents are not specific for cancer cells and also kill rapidly dividing normal cells, such as epithelial cells that line the digestive tract. The resulting toxicity of these drugs limits their effectiveness, and many cancers are barely eliminated by doses of chemotherapy that can be tolerated by the patient. Consequently, although major progress has been made in cancer treatment, nearly half of all patients diagnosed with cancer ultimately die of the disease [1].

### Cells are Programmed to Die When Needed

Apoptosis is a process of programmed cell death whereby cells under stress such as mutatory lesions in the genetic material or viral infections, undergo self destruction to arrest the propagation of their harmful effects to the rest of organism [1]. This is accomplished by means of specific proteases known as caspases. Normally, these proteases exist in an inactive state where they are known as procaspases [2]. An apoptotic signal causes the aggregation of procaspases, resulting in a conformational change, leading to their activation to caspases [2]. The active caspases initiate a cascade of events which ultimately leads to cell death.

There are two pathways that are followed by the apoptotic signal (*Figure 1*).

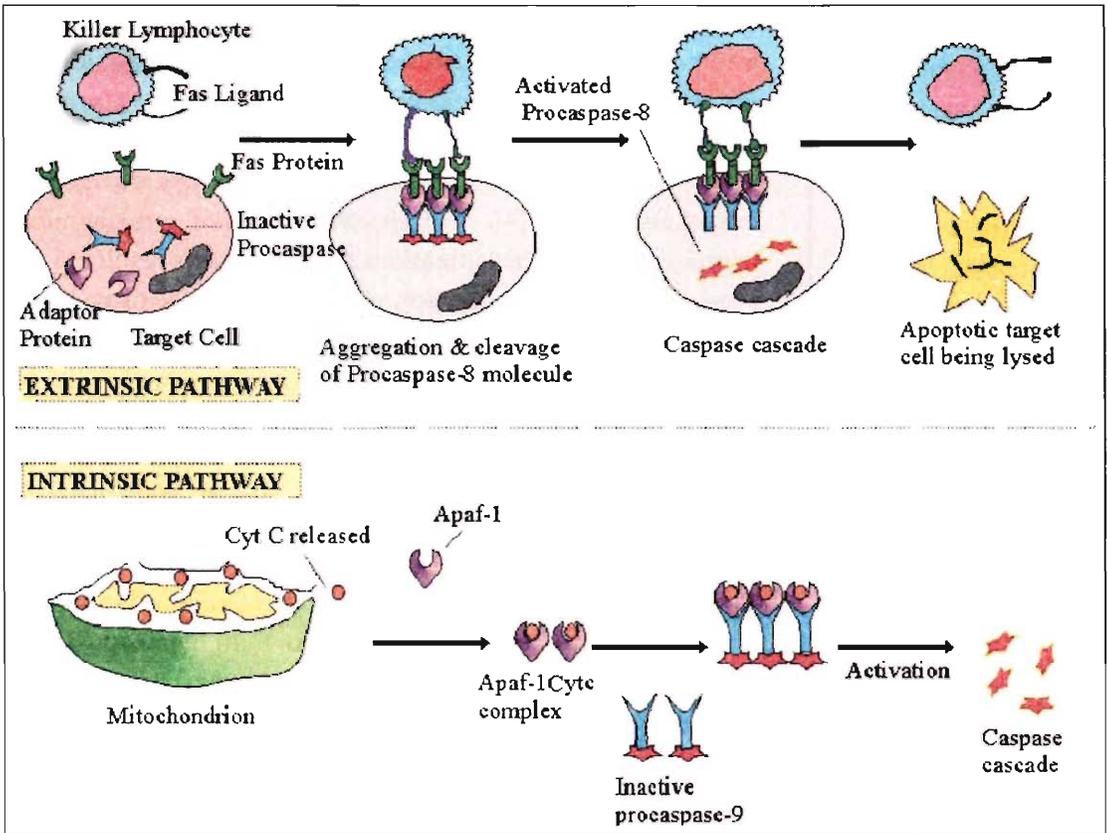
**Extrinsic Pathway** is induced by the binding of the Fas ligand on the surface of killer lymphocyte of the immune system to the Fas receptor of the target cell. This recruits the adaptor protein to the membrane, leading to the aggregation and cleavage of procaspase 8 molecules and ultimately to cell death [2].

**Intrinsic Pathway** is induced by the release of Cyt c from the mitochondria into the cytosol by an intracellular apoptotic signal that induces stress in the mitochondria. The Cyt c thus released binds and activates the cytosolic adaptor protein Apaf 1 that causes the aggregation and activation of procaspase 9 molecules. Activated caspase 9 then activates another inactive procaspase known as procaspase 3 that leads to cell death [1,2].

#### Keywords

Apoptosis, cytochrome c, proteasome, cancer.





## Cancer Cells Evade Apoptosis

Apoptosis is an integral part of the differentiation program of many cell types. Cancer cells fail to undergo apoptosis and therefore exhibit increased life spans compared to normal counterparts. Following DNA damage, apoptosis is induced in normal cells while it is blocked in cancer cells. This contributes to the resistance of cancer cells to irradiation and many therapeutic drugs that act by damaging DNA [1]. The characteristic failure of cancer cells to undergo apoptosis also contributes substantially to tumor development. We have targeted this characteristic of cancer cells and propose that if this obstruction placed by cancer cells to the normal apoptotic process is removed, it may lead to a cure for cancer.

Before we advance further, let us first understand the basis

*Figure 1. Schematic depiction of apoptotic pathways, whereby procaspases are activated ultimately leading to cell death. In extrinsic pathway, procaspase activation is triggered from outside the cell by activation of death receptors on the cell surface. In intrinsic pathway, procaspase aggregation and activation is triggered from within the cell, mitochondria are induced to release Cyt c that bind with an adaptor protein called Apaf-1 and results in apoptosis.*

The elevated levels of mitochondrial bound isoforms of hexokinase in the tumor cells results in the evasion of apoptosis, thereby allowing the cells to continue proliferating.

behind the arrest of the apoptotic process in cancerous cells. It is well known that the cytosolic enzyme hexokinase catalyzes the initial step of glycolysis, the conversion of glucose to glucose-6-phosphate) [3]. In tumor cells there is an over production of isoforms of hexokinase (HK-I and HK-II) and they are bound to mitochondria [3], [4]. The elevated levels of mitochondrial bound isoforms of hexokinase in the tumor cells results in the evasion of apoptosis, thereby allowing the cells to continue proliferating [5]. The enzyme binds to the mitochondrial outer membrane via its hydrophobic amino acid residues at the N-terminal, preventing  $\text{Ca}^{2+}$  dependent opening of the mitochondrial permeability transition pore (PTP) and release of proapoptotic protein Cyt c (apoptotic signal), thus arresting apoptosis.

In our scheme we have proposed the use of engineered viruses and proteasome as potential tool in fight against cancer. Proteasome is an intracellular protein chopper. It is an abundant ATP dependent protease constituting nearly 1% of the cellular protein. Present in the cytosol and nucleus, it consists of a central hollow cylinder (20 S) formed of four heptameric rings. Two  $\beta$  rings each made up of seven subunits form the central catalytic core, surrounded on either side by rings made up of seven subunits. Each end of cylindrical core is attached to a large protein complex (19 S cap) containing approximately twenty distinct polypeptides which include at least six proteins that hydrolyze ATP. 19 S cap unfolds the proteins to be digested after recognizing them via the ubiquitin tag [2]. The ubiquitin pathway<sup>1</sup> involves a complex of three enzymes  $E_1$ ,  $E_2$  and  $E_3$ .  $E_1$  is an ATP dependent ubiquitin activating enzyme.  $E_2$  is conjugating enzyme that transfers the ubiquitin from  $E_1$  to the target protein along with the help of  $E_3$ .  $E_3$  binds to specific degradation signals in a protein substrate and helps  $E_2$  to form a multiubiquitin chain linked to the lysine residue of the substrate [2].

Proteasomes are involved in the degradation of misfolded polypeptides. A degradation signal can be created in a protein

<sup>1</sup>See article, The Nobel Prize in Chemistry 2004: 'Ubiquitous' quality control of life, *Resonance*, Vol.10, No.1, p.41, 2005.



causing its rapid ubiquitilation and subsequent destruction by the proteasome. Three common ways suggested for this are:

1. Phosphorylation of a specific site on a protein that un-masks a normally hidden degradation signal.
2. Unmasking a signal by the regulated dissociation of a protein subunit.
3. Creation of powerful degradation signals by a single cleavage of a peptide bond in the target protein provided that this cleavage forms a new N-terminus that is recognized by specific  $E_3$  as a destabilizing N-terminal residue [2].

### N-end Rule of Yeast

There are twelve destabilizing residues (preferentially recognized by an specific  $E_3$ ) arginine, lysine, histidine, phenylalanine, leucine, isoleucine, tyrosine, tryptophan, aspartate, glutamate, asparagine, and glutamine, out of twenty standard amino acids. These are recognized by a special ubiquitin ligase that is conserved from yeast to humans [2].

### A Possible Scheme for Arresting Cancer

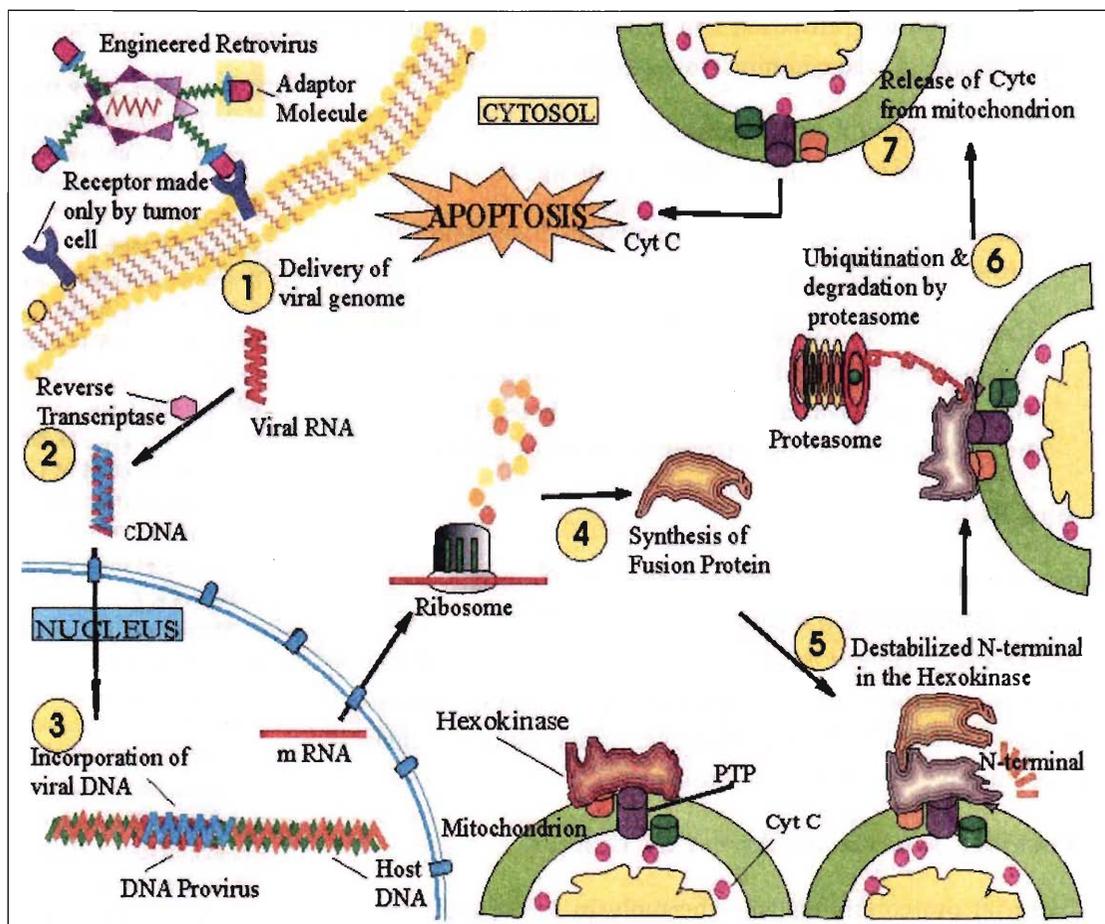
A bacterial proteolytic enzyme thermolysin has specificity to cleave on the amino terminal (N) side of leucine, isoleucine, phenylalanine, tyrosine and tryptophan [6]. All the five residues match the specificity of the N-end rule and can create a destabilizing N-terminal which can be recognized as a potential signal for degradation by the proteasomal pathway. A possible scheme involving thermolysin for checking cancer is outlined in *Figure 2*.

A gene coding for a fusion protein having both the catalytic activity of thermolysin as well as a conformation-specific ligand to hexokinase can be delivered to the cancerous cells through engineered retroviruses (Step 1). Taking into account the small packaging capacity of the viruses, only the smallest possible fragment of the thermolysin gene could be taken that can sustain

### Suggested Reading

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**Figure 2.** A possible scheme for arresting cancer.

its catalytic activity. The gene for this chimeric protein would be packaged into the viral genome under the regulation of an efficient promoter that can facilitate its desired expression.

This type of delivery using retroviruses is being traditionally employed in gene therapy to splice a functioning copy of a gene into the body of a patient in whom that gene has ceased to work properly [7]. Scientists have engineered viruses that selectively target the cancerous cells by attaching adaptor molecules on the viral outer coat protein which can recognize the altered surface receptor of the cancerous cells not found on normal cells [7].

The retroviral genome (RNA) in the cytosol of the cancerous cell will be acted upon by an enzyme called reverse transcriptase,

which is carried by the virus itself, and a corresponding DNA copy of the RNA is formed (Step 2). This viral DNA will move into the nucleus and will get incorporated into the host genome (like normal retroviral infection) (Step 3). The chimeric protein when expressed (Step 4) will specifically target (as it contains conformational ligand to hexokinase):

- i) Mitochondrial bound hexokinase.
- ii) Newly translated hexokinase .

Action of thermolysin will create a destabilizing N-terminal in the hexokinase (Step 5) leading to its ubiquitilation and subsequent degradation by proteasome (Step 6).

The proposed scheme will have three-fold attack on cancerous cells.

**I.** Cancerous cells will be deprived of their energy demand that is largely fulfilled by anaerobic glycolysis. This will arrest the cancer cell proliferation.

**II.** Removal of the hexokinase from the outer mitochondrial membrane will open the channel that responds to the apoptotic signals. This will induce mitochondria to release Cyt c leading to the death of cancerous cell by apoptosis (Step 7).

**III.** Apoptotic signals will induce provirus transition to lytic cycle. Progeny will continue to target other cancer cells in an amplified manner.

## Summary

Cancerous cells evade apoptosis and thereby continue proliferating. They do so by preventing the release of proapoptotic protein Cyt c from the mitochondria. Engineered viruses can be used to deliver a gene that will code for a protein that will clear the obstacle in the release of Cyt c by mitochondria using a hybrid proteolytic enzyme.

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