The ATP Site of Protein Kinases as Target for Drug Development: From Natural Compounds to Gleevec

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My personal involvement in the story of protein kinases as targets for drug development began in the laboratory of the late Ef Racker at Cornell University in 1973. As a young postgraduate fellow I was involved in research that was based on the idea that the so-called uncoupled Na^+/K^+ adenosine triphosphatase activity is the reason for the high lactic acid production in Ehrlich ascites tumor cells. At this time it was shown in the laboratory of Ef Racker that quercetin, a natural compound that exists in many fruits and vegetables, inhibits both Na^+/K^+ ATPase activity and lactic acid production in these cells [1]. Thus, the concept was that quercetin is a natural coupling factor of uncoupled ATPases. After one year of hard work on this concept I came up with a different idea: namely, quercetin is a compound that inhibits many enzymatic systems that use ATP as a substrate. Indeed, we demonstrated that quercetin is a strong inhibitor of hexokinase, the first enzymatic activity in glycolysis [2]. Interestingly, quercetin inhibits only the active hexokinase, which is bound to the mitochondrial membrane. Soluble hexokinase from the tumor cells or a commercially obtained one was totally unaffected by quercetin. These observations led me to the following conclusions: a) high activity of hexokinase may be at least part of the reason for the high aerobic glycolysis in Ehrlich ascites tumor cells, and b) quercetin needs the specific configuration or domain of a kinase activity in order to exert its effect.

The inhibitory effect of any drug on the proliferation of malignant cells needs to demonstrate an inhibitory effect on DNA, RNA and protein synthesis. Indeed, my colleagues and I in Israel demonstrated that quercetin is a strong inhibitor of the synthesis of these macromolecules.

The next question that we asked ourselves was how does this flavone control so many enzymatic functions in proliferating cells? Should it interact with regulatory enzymatic systems, which control the metabolism of the cells? Our observations demonstrated that quercetin increases the cAMP level due to inhibition of cAMP-phosphodiesterase activity. The next obvious question that intrigued us was: is quercetin an inhibitor of PK activity? The surprising and interesting answer that came from our observations was that this drug inhibits partially purified cAMP-independent protein kinases but not the cAMP-dependent PK activity [3]. In order to extend our knowledge on the effect of quercetin on cAMP-independent PK activity I joined the laboratory of Ray Erikson at Denver that discovered the *src* gene protein product, $pp60^{v\text{-}src}$ [4], and its activity as cAMP-independent PK [5]. The tyrosine phosphorylation activity of this protein, first described by Eckhart et al. [6], became the leading subject of my research in the laboratory of Ray Erikson. Using both immuno-affinity and sequential columns purified with $pp60^{v\text{-}src}$ we were able to characterize the trinucleotide-binding domain towards ATP and GTP of $pp60^{v\text{-}src}$ [7]. Finally, having these tools and knowledge, we were able to demonstrate that quercetin is an efficient drug that inhibits the tyrosine phosphorylation of $pp60^{v\text{-}src}$ by interaction with the ATP site of the enzyme. Interestingly, using several physiologic protein substrates we found that the purified catalytic

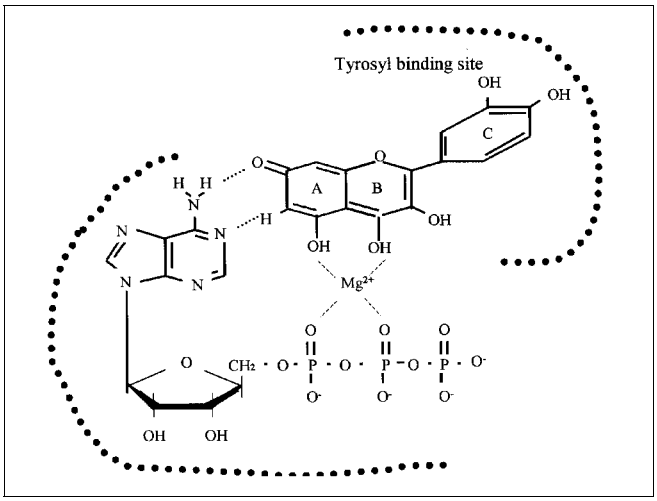

Figure 1. ATP-quercetin transition model in the tyrosine kinase functional sites.

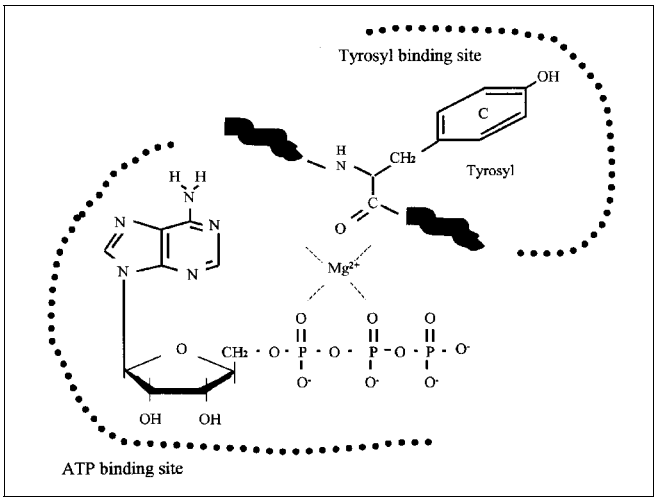

Figure 2. ATP-protein substrate transition model in the tyrosine kinase functional sites.

subunit of the cAMP-dependent PK was unaffected by this flavone [8].

These were the first observations in the literature that: a) a natural drug that inhibits many of the biochemical functions of malignant cells in tissue cultures is an efficient inhibitor of tyrosine kinase activity, b) the inhibitory function of this flavone is due to interaction with the ATP site of the $pp60^{v\text{-}src}$, and c) quercetin is able to sense the structural differences between the ATP site of the catalytic subunit of the cAMP-dependent PK and the ATP site of the $pp60^{v\text{-}src}$. Thus, the ATP functional sites of protein kinases respond in different ways to relatively specific drugs. These observations were the first to describe a possible small molecule structure that interacts with the specific ATP catalytic sites of tyrosine kinases.

Further extension of these observations came from the discovery in 1987 by Akiyama and co-workers [9] that genistein, an isoflavone extracted from soybeans, is a much more specific inhibitor towards tyrosine protein kinases. Following our findings, Akiyama et al. demonstrated that genistein interacts with the ATP site of several tyrosine kinases, including the epidermal growth factor receptor tyrosine kinase activity [9].

Tyrosine kinase inhibitors such as resveratrol, which associate with the ATP site of the enzyme, were found also in red wine [10], and probably many of them are included in the large family of polyphenols that are naturally produced by plants and microorganisms. It seems logical that these natural products, and their function as inhibitors of tyrosine kinase activity, are among the factors contributing to the chemopreventive role of vegetables and fruits in cancer development.

In 1989, Dr. J.A. Hickman from Birmingham asked me to present data from my work on the inhibition of tyrosine kinases by flavones, under the title: "The cell membrane and cell signals as targets in cancer chemotherapy," at a meeting organized by the American, British and European Organizations for Cancer Research. He particularly asked me to address the main problems of specificity associated with flavones being competitive and selective inhibitors that interact with the ATP sites of tyrosine protein kinases. I disclosed my views, based on the experimental evidence that the flavones compete with ATP at the trinucleotide-binding site of the tyrosine kinases [8,9]. I designed the hypothesis that ATP can form, with the flavones, a complex stabilized by hydrogen bonds and $Mg^{2+?}$ ions at the tyrosine kinase catalytic domain of the enzyme. This complex is formed between the hydroxyl group at the A ring of the flavone and the NH_2 in the heterocyclic group of the trinucleotide. The phenyl group of the flavone (ring C) that resembles the phenyl group of the tyrosine associates with the binding domain of tyrosine in the protein substrate site [Figure 1]. My original suggestion was that the hydrogen-bonded complex of the flavones and ATP mimic the transition state of the trinucleotide and the phenyl group of the tyrosyl residue of the protein substrate in the tyrosine kinases catalytic domain [Figure 2]. This presentation was summarized in a section of two special articles dedicated to this meeting [11,12].

Are the ATP sites of tyrosine kinases and the hydrogen-bonded complex of the ATP-flavone a model for the design of drugs for cancer chemotherapy? Designing drugs that mimic the structure of ATP or interact with the ATP site at the catalytic domain of tyrosine kinases has many logical limitations. First, the concentration of ATP in the majority of cells is in the range of 5 mM or higher. Thus, high specificity of the drug to the enzymatic site will be required in order to make this drug effective. Secondly, the sequence of the ATP-binding site of kinases is highly conserved. Thus, the possibility of selectively differentiating between the ATP-binding sites of protein kinases with specific drugs seems to be almost a mission impossible.

Taking into consideration these limitations, in 1988 Alex Levitzki from the Hebrew University designed the synthetic tyrosine kinase inhibitors – the tyrphostins [13]. The first of three criteria that guided his research was the following: The compounds should be competitive with the (protein) substrate of EGFRK (a tyrosine kinase receptor) and not with adenosine triphosphate. The PTK (protein tyrosine kinase) inhibitors quercetin and genistein, which compete with ATP, inhibit other protein kinases and are highly cytotoxic. Nevertheless, in 1995, these views were changed and became more receptive to the possibility that the ATP site of

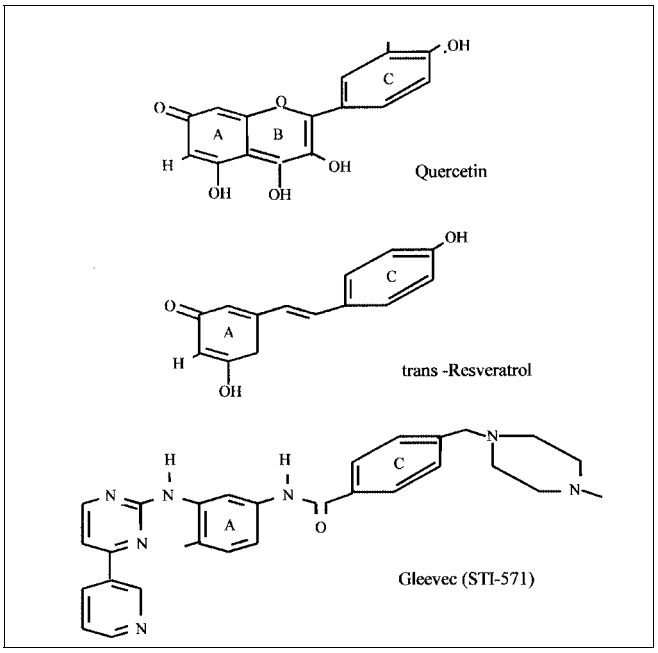

Figure 3. Structures related to Gleevec (STI-571).

tyrosine kinases can be specific, and hence, a possible target for drug design for cancer chemotherapy [14].

Did the ATP site of tyrosine kinases become a major site for development of drugs against cancer? Recently, the Swiss drug company Novartis successfully used the concept to design an efficient drug that interacts with the ATP site of the platelet-derived growth factor receptor tyrosine kinase activity. STI 571 – under the commercial name of Gleevec [Figure 3] – became the proof of the concept that tyrosine kinase inhibitors oriented towards the catalytic ATP site of the *bcr-abl* gene product have high clinical efficacy and tolerability in chronic myelogenous leukemia patients [15,16].

The ATP site of the tyrosine kinase catalytic domain of oncogene protein products, such as *src* and *bcr-abl* or growth factor receptors, is only the first step in the future design and production of drugs that will use the signal-transducing machinery in cells for the development of new and specifically oriented drugs. In this regard, drugs that will be oriented towards specific domains in proteins that are responsible for the transmission of the signals within the cell will be at top priority for future development. Specific sites for protein-to-protein interaction, such as SRC homology 2 (SH2) or 3 (SH3) domains, will be suitable for such drug developments.

The proteins that possess tyrosine kinase activity are only a small part of signal-transducing proteins that will become targets for drug development. In a review by Wolf and Seger in this issue of *IMAJ* [17], Figure 1 shows that additional protein kinases, such as proteins from the mitogen-activated protein kinase family may become highly important targets for drug development aimed at proliferating cells. In this regard, the ATP site at the catalytic domain of MAP kinases may differ from the ATP site of other protein kinases during the creation of the active transition state configuration of these enzymes. In addition, the regulatory site of these enzymes, which consists of the phosphorylated form of the amino acid residues Thr-X-Tyr in the active form of the enzyme, may become the specific site for drug mimetics and enzyme activity reduction. Additional sites for drug action can be the specific domains of the MAP kinases, which are responsible for the translocation of these enzymes to the nucleus. Thus, specific strategies for drug development can be employed for the cessation and the transmission inhibition of the signaling process within cells under pathophysiologic conditions in different diseases.

In conclusion, the signal transduction mechanism of cells under disease conditions can provide future drug designers with ample opportunities to develop sophisticated drugs that will prevent the transmission of abnormal signals to specific cellular enzymatic targets.

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