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News and Perspectives

From discovery of tyrosine phosphorylation to targeted cancer therapies: The 2018 Tang Prize in Biopharmaceutical Science

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ABSTRACT

Protein tyrosine kinases (TKs) are a family of enzymes that catalyze the phosphorylation of proteins at tyrosine residues. TKs play key roles in controlling cell growth and many other functions by modulating the status of tyrosine phosphorylation of regulatory proteins critical for numerous cellular signaling pathways. Dysregulation of TKs caused by genetic abnormalities (mutation, amplification, fusion, etc.) results in uncontrolled cell growth, and ultimately leads to cancer. Thus, identification of dysregulated TK(s) in a specific cancer type and development of TK inhibitors (TKIs) that can potently block activity of the dysregulated TK establish the foundation of modern targeted cancer therapies. The 2018 Tang Prize in Biopharmaceutical Science was awarded to Tony Hunter as well as Brian Druker and John Mendelsohn for their great contributions in discovering oncogene src as a TK and developing small molecule TKIs or therapeutic monoclonal antibodies against receptor TK, respectively.

Protein phosphorylation is a biological reaction that involves transfer of a phosphate group from ATP to specific amino acid residue(s) of a protein, which is catalyzed by a big family of enzymes called protein kinases. There are >500 protein kinases encoded in the human genome [1]. Serine, threonine and tyrosine are the three major amino acid residues in proteins that can be phosphorylated by protein kinases in eukaryotic cells, with an estimated ratio of 1000:100:1 [2]. Phosphorylation of proteins (or enzymes) at specific amino acid residue(s) can alter their 3D structures and thus modulate their biological functions (or activities). The importance of protein phosphorylation as a biological regulatory mechanism to control a particular physiological function came from the pioneering studies by Edmond H. Fischer and Edwin G. Krebs on the hormone-dependent glucose metabolism in the early 1950s [3]. Soon after, numerous researchers confirmed the critical role of protein phosphorylation in modulating diverse biological processes other than glucose metabolism. However, only serine and/or threonine phosphorylation of proteins could be observed in all these prior studies. At that time,

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nobody knows if a protein (or enzyme) can be phosphorylated on tyrosine residue(s), not to mention the biological meaning of protein tyrosine phosphorylation.

Discovery of protein tyrosine phosphorylation

The first discovery of protein tyrosine phosphorylation was made by Tony Hunter's lab in 1979, who found an activity phosphorylating tyrosine in the immunoprecipitates of the animal tumor virus transforming protein polyoma T antigen [4]. In the next few years (1980–1984), Hunter and other scientists quickly demonstrated that both v-Src (the Rous sarcoma virus transforming protein) and epidermal growth factor receptor (EGFR) possess intrinsic TK activity, and EGF can induce rapid tyrosine phosphorylation of proteins in A431 human tumor cells [5–9]. These seminal findings prompted other researchers to demonstrate the intrinsic TK activity of additional growth factor receptors, such as PDGF receptor and insulin receptor, in the 1980s. By this time, researchers began to realize that ligand-induced tyrosine phosphorylation can be a major and common mechanism for the transmission of signals across the plasma membrane.

The finding that v-Src had TK activity strongly indicated uncontrolled tyrosine phosphorylation as a potent transformation mechanism. Immediately, Hunter and his colleague's 1980 report showed the precise correlation between TK activity of v-Src from temperature-sensitive transforming mutants of Rous sarcoma virus and their transforming potential in mouse cells, providing direct evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus [10]. Quickly, researchers investigating the BCR-ABL fusion protein, a human oncogene resulting from the fusion of the BCR gene with the c-ABL TK gene in chronic myelogenous leukemia (CML), found that BCR-ABL had increased TK activity (in 1984/1986) [11,12] and caused CML in mice (in 1990) [13–16]. Subsequent search for human tumor oncogenes identified many additional human TK mutants, and several of these are mutant forms of receptor TKs, such as KIT in gastrointestinal stromal tumors (in 1998) [17,18] and EGFR in lung cancer (in 2004) [19–21]. ERBB2, another transmembrane TK was observed to be frequently overexpressed in breast cancer (between 1987 and 1992) [22–24].

Development of tyrosine kinase inhibitors

Through the understanding of aberrant tyrosine phosphorylation caused by viral or cellular oncogenes as one of the major causes of cancer, the Hunter's pioneering work inspired other researchers to develop small molecule inhibitors of oncogenic TKs targeting the ATP binding site, with the hope that they might ultimately be useful in cancer therapy. The development of tyrphostins (tyrosine phosphorylation inhibitors) by Alex Levitzki in 1988 represents the first attempts at rational design of TKIs in academia, in which the most potent tyrphostins effectively blocked the EGF-dependent proliferation of A431 cells with little or no effect on the EGF-independent proliferation of these cells [25]. Their later studies further showed that some tyrphostins were selective inhibitors of BCR-ABL, which induced the K562 CML cell line to terminally differentiate into nondividing erythroid cells [26,27]. In the pharmaceutical industry, CIBA-Geigy (now Novartis) initiated a TKI program in 1986 led by Nick Lydon, focusing first on the PDGF receptor to develop a series of 2-phenylaminopyrimidine derivative TKIs. Brian Druker, a physician scientist who had been working on CML and started treating CML patients since the early 1990s, made up his mind to find a better way for treating this disease. He collaborated and worked closely with the team of the TKI program at CIBA-Geigy, identifying CGP57148B (a 2-phenylaminopyrimidine derivative) as a potent and relatively selective inhibitor of v-Abl (the oncoprotein of the Abelson murine leukemia virus) [28] in 1996 and further demonstrating that CGP57148B could inhibit the growth of CML cells and BCR-ABL–transformed cells both in culture and in mice [29,30]. Based on these encouraging data, Druker and Charles Sawyers initiated in June 1998 a series of phase I/II clinical trials in CML patients, demonstrating the effectiveness of imatinib (the generic name of CGP57148B, also known as STI571 or Gleevec/Glivec) in treating chronic phase CML [31–33]. Due to these successful trial results, the United States Food and Drug Administration (FDA) approved imatinib for the treatment of CML on May 10, 2001. Moreover, as imatinib is also a potent inhibitor for the c-KIT receptor tyrosine kinases reported to be mutated/activated in gastrointestinal stromal tumor (GIST) [34], clinical trials have shown that imatinib was quite effective in GIST patients [35], leading to the FDA's approval of imatinib in treating GIST in February 2002.

Development of anti-EGFR monoclonal antibodies

In addition to targeting the TK's ATP binding site, blockage of the receptor TK's transmembrane signaling represents another approach to inhibit TK activity in cancer. Beginning from 1983, John Mendelsohn, Gordon H. Sato and their collaborators reported the production of murine monoclonal antibodies (mAbs) 528 and 225 against the extracellular domain of EGFR, which could inhibit human cancer cell proliferation by blocking the transduction of EGFR signaling in culture and in athymic mice [36,37]. Subsequent preclinical studies in the Mendelsohn's lab characterized the molecular events involved in mAb-mediated receptor internalization, mechanisms cause the cell growth inhibition, as well as additive effects of combining anti-EGFR mAb therapy of human tumor xenografts with chemotherapeutic agents [38]. In 1991, the initial phase I clinical trial was performed with indium–III–labeled murine mAb 225 in patients with advanced lung cancer [39]. After that, a chimeric human:murine version of mAb 225 called C225 (chimeric 225, cetuximab) was created to obviate an immune response to repeated doses, which was licensed to ImClone Systems in 1994 for clinical trials. Subsequently, Bristol-Myers Squibb and Merck KGaA joined with ImClone for a series of trials in the United States and Europe, respectively, and cetuximab was approved by the U. S. FDA for treatment of colon cancer in 2004 and for head and neck cancer in 2006 [40,41].

Perspectives

Since the discovery of tyrosine phosphorylation and the first TK in 1979 by Hunter, it took more than 20 years for the first cancer drug (imatinib) that acts against a specific TK (BCR-ABL) to be approved for clinical use. Currently, more than 30 small molecule TKIs or therapeutic mAbs against protein kinases have been approved for cancer treatment [42], and dozens of protein kinase inhibitors are in cancer clinical trials, including several directed against serine/threonine kinases implicated in cancer. Apparently, the pioneering work from Hunter, Druker and Mendelsohn all together establish the foundation of modern targeted cancer therapies. In spite of the tremendous progress in targeted cancer treatment, significant challenges still remain. For example, development of resistance to TKI or anti-EGFR therapy is usually observed in patients, for which additional strategies are needed to overcome the resistance [38,43]. Moreover, due to the extremely complex nature of cancer, it is still hard to precisely select patients most likely receive benefit from TKI or anti-EGFR therapy. The use of modern multi-omics (genomics, transcriptomics, proteomics, and metabolomics, etc.) techniques in combination with bioinformatics and systems biology approaches has allowed the in-depth interrogation of clinical samples, which should greatly enable researchers to identify novel genes and signalling networks involved in determining the responsiveness of tumors to a specific drug treatment, as well as the molecular signatures and genotypes/proteotypes/metabolotypes that predict responses to certain drugs.

Conflicts of interest

The author declares that he has no competing interest.

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