Commentary

Hunting hidden pieces of signaling pathways in hepatocellular carcinoma

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Advanced hepatocellular carcinoma (HCC) has a poor prognosis with limited treatment options. During the last decade, sorafenib, a multi-tyrosine kinase inhibitor (TKI), has been the only available systemic agent for first-line treatment of unresectable HCC (1). Recently, another multi-TKI, lenvatinib, was added to the list of first-line treatment alternatives based on the results of the phase III RREFLECT trial. For second-line treatment, two multi-TKIs, regorafenib and cabozantinib, were approved in 2016 and 2018, respectively. Among other targeted therapies, ramucirumab and an anti-PD-1 antibody, nivolumab, will soon be added in a second-line setting to the therapeutic armamentarium. These agents, however, generally lead to disease stabilization rather than tumor shrinkage. Achievement of complete remission with available systemic treatments still remains rare, however, and therapeutic breakthroughs are still needed.

A research group at the University of Basel, Switzerland, led by Michael N. Hall and researchers from the Salk Institute led by Tony Hunter together reported a phospholysine phosphohistidine inorganic pyrophosphate, LHPP, as a tumor suppressor for liver cancer in the March 29, 2018 issue of *Nature* (2). They demonstrated for the first time that previously overlooked form of post-transcriptional modification (PTM), known as histidine phosphorylation, holds the key to the development of HCC. Upregulation of the mTOR pathway is observed in 40–50% of HCC patients, and is also associated with poor prognosis as well as the resistance to sorafenib (3-5). In this comprehensive study, they employed liver-specific double-knockout (L-dKO) mice lacking two major tumor suppressors in the mTOR pathway, PTEN and TSC1, thereby causing constitutive activation of PI3K/AKT/mTOR signaling. This mTOR-driven HCC mouse model consequently develops hepatomegaly at 6 weeks of age and advanced liver tumors by 20 weeks. Quantitative proteomic analysis of 12 tumors obtained from three mice was compared with liver proteins extracted from six age- and sex-matched control mice. This revealed that 17 kinases were upregulated in at least 10 tumors. Two of these kinases were NME1 and NME2, the only mammalian histidine kinases reported to date. Coincidentally, LHPP was one of the four phosphatases specifically downregulated in the liver tumors. Further investigation confirmed that LHPP was indeed a protein histidine phosphatase that was significantly decreased in L-dKO tumors than in non-tumor liver tissue, thereby globally augmenting histidine phosphorylation (pHis) in the tumor. Consistent with this finding, decreased immunohistochemical expression of LHPP was observed in clinical samples of HCC tissue, and low levels of LHPP mRNA were correlated with poor prognosis. It was therefore concluded that LHHP is a tumor suppressor, demonstrating the importance of histidine phosphorylation in cancer development.

Despite accumulating evidence that histidine phosphorylation plays a crucial role in the regulation of cellular signaling in prokaryotes and lower eukaryotes, research on pHis in mammalian cells has lagged far behind that of phosphoserine (pSer), phosphothreonine (pThr) and phosphotyrosine (pTyr) due to its acid-labile and heat-sensitive nature and the long-standing dearth of suitable methods and reagents such as sequence-independent pHis antibody (pan-pHis antibody) (6). One unique feature of pHis is that it is phosphorylated at either the N-1 or N-3

nitrogen of the imidazole ring, which generates two isomers, 1-pHis and 3-pHis. To allow precise characterization of the cellular function of pHis, two properties of pan-pHis antibodies are essential: (I) an ability to differentiate between 1-pHis and 3-pHis isomers, and (II) no cross-reactivity with pTyr. Prior to their analysis of liver tumors, Hunter's team succeeded in developing highly specific and isoform-specific monoclonal antibodies (mAbs) against 1-pHis or 3-pHis by immunizing rabbits with peptide libraries containing stable analogues of pHis isomers, the phosphoryl-triazolylalanine analogs (1-pTza and 3-pTza) (7). Application of these antibodies to analysis of L-dKO mice revealed a potential key role of histidine phosphorylation in HCC development, as described above. This study undoubtedly opened another promising path to future cancer therapies, reminiscent of Hunter's ground-breaking discovery of the first known tyrosine kinase, Src, back in 1980. At that time, not many biomedical scientists had paid much attention to pTyr. This discovery led to the development of kinase inhibitors that were subsequently used for the treatment of cancer and other diseases. As of July 2018, the United States Food and Drug Administration (FDA) had approved 48 small-molecule kinase inhibitors, 41 of which are for cancer treatment, as exemplified by the BCR-ABL1 inhibitor imatinib that has revolutionized the treatment of chronic myeloid leukemia (CML).

Although the study identified LHHP as a tumor suppressor, restoration or reactivation of tumor suppressors in HCC patients is still challenging from a therapeutic viewpoint. In this context, development of small molecules that can restore or reactivate tumor suppressor function may be a more productive avenue. Finally, Hunter's team searched for potential LHHP targets preferentially expressed in tumor-derived cells, and identified 9 histidine-phosphorylated proteins including ACLY (ATP citrate lyase) previously reported to be phosphorylated (7). Further elucidation of the biological roles of these proteins, their interacting proteins and downstream effectors may lead to the discovery of as yet unknown pieces of therapeutically relevant signaling pathways, including histidine kinases that could be potential therapeutic targets for HCC. It is worth noting that immunofluorescent staining of cancer cell lines with anti-3-pHis mAb revealed specific staining in mitotic structures, reflecting that pHis protein(s) regulates the cell cycle (8). Elevated histone H4 histidine kinase activity has been observed in regenerating rat liver and biopsy specimens of human HCC. Together, these findings suggest the presence of as yet unidentified pHis proteins with oncogenic properties in HCC.

Future refinement of both methods and tools including phosphohistidine kinases and phosphatase inhibitors will accelerate research on histidine phosphorylation in various types of cancer. Generation of a complete list of pHis substrates by immunoaffinity purification of pHis mAbs along with liquid chromatography tandem mass spectrometry (LC-MS/MS) will lead to the development of sequence-specific pHis antibodies. As the number of such antibodies increases, antibody-based large-scale pHis proteomic analysis with high sensitivity will become feasible, leading to the development of biomarkers and diagnostics that are necessary for precision medicine. The next decade is likely to see clinical trials of therapeutics targeting pHis proteins, histidine kinases or phosphatases, heralding an exciting new era of research on signaling transduction in cancer.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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