Synthetic Lethality in the Search for Novel Molecular Targets in Cancer Therapeutics

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Cancer treatment methods based on suppressing the function of a specific target molecule that is mutated or overexpressed are defined as "molecular targeted therapy". In molecular targeted therapy, the specific targets are identified in the drug discovery and treatment design stages. Many molecular targeted therapy methods are currently used for cancer treatment, and the group of molecular targeted drugs has grown far beyond the classical chemotherapeutic drug family. However, the design of targeted agents for certain molecules such as tumor suppressor genes, which also play a causative role in cancer, is complex. Here, I briefly review the history of molecular targeted drugs and introduce a search using synthetic lethal phenotypes as one method to identify molecular targeted drugs against tumor suppressor gene mutations.

**Key words**: targeted cancer therapy, synthetic lethal interactions

**Introduction: classical chemotherapy drugs**

Cancer is characterized by the uncontrolled growth and metastasis of cells due to genetic abnormalities. The uncontrolled growth of cancer cells affects organ function. Cancer has been the leading cause of death in Japan since 1981. The development of anticancer drugs began with the development of mustard gas, a chemical weapon, during World War II. Mustard gas-induced damage is caused by inhibition of cell division, a function similar to that of radiation, which led to the idea of using it to treat cancer. Later, anticancer drugs called alkylating agents, such as the nitrogen mustard derivative cyclophosphamide, were developed. In the subsequent 40 years, the most widely used anticancer agents were defined as cytotoxic drugs. Cancer cells divide at a faster rate than normal cells, and are therefore more sensitive to cytotoxic anticancer drugs. In addition to the alkylating agents described above, cytotoxic drugs inhibit enzymes that control the cell cycle and DNA replication, such as topoisomerase inhibitors and microtubule inhibitors. The discovery of metabolic pathways specific to cancer cells led to the development of antimetabolites.

**Emergence of molecular targeted drugs**

Studies of viral carcinogenesis, which began in the 1960s, led to the discovery of oncogenes, and research into the genetic background of cancer led to the discovery of tumor suppressor genes. Since the 1980s, advances in molecular biology have improved our understanding of the relationship between cancer and genes and resulted in the identification of oncogenes involved in the development, proliferation, and metastasis of cancer cells. In addition, the causative roles of gene mutations, deletions, duplications, and translocations in cancer were clarified. Molecular targeted drugs are designed to target specific gene alterations that distinguish cancer cells from normal cells. The notion that cancer is an abnormality of cell
differentiation became widely accepted, and the efficacy of differentiation inducers was demonstrated. Furthermore, the involvement of cancer microenvironments including blood vessels, fibroblasts, and immune system cells in the formation of solid tumors demonstrated that they are essential for tumor growth. Molecular targeted agents include those that shrink tumors by targeting the tumor microenvironment. Immune surveillance is thought to be involved in the mechanism of action of cancer therapeutic agents that modulate the immune system.

Molecular targeted drugs act on molecules that are absent from or do not function in normal cells, and their cancer cell selectivity and safety are superior to those of cytotoxic drugs. Initially, these drugs were not thought to elicit a tumor-reducing effect because they only suppressed the growth of cancer cells. However, certain molecularly targeted drugs result in dramatic tumor shrinkage and unexpected toxicity. Molecular targeted agents are mostly monoclonal antibodies and low molecular weight compounds, although there are other types of drugs. The first molecular targeted drug used in clinical practice was a monoclonal antibody.

Trastuzumab (a monoclonal antibody)

Trastuzumab was the first monoclonal antibody approved for clinical applications in 1998 and the first molecular targeted drug with proven survival benefit in women with human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer. HER2-positive breast cancers tend to grow faster and are more likely to metastasize and recur than HER2-negative breast cancers, and they are therefore associated with poor prognosis. Although HER2 is also expressed in normal cells, it is overexpressed or dysregulated in breast cancer. The HER2 gene is amplified in up to 25% of all breast cancers. HER2 is a transmembrane receptor tyrosine kinase that belongs to the epidermal growth factor receptor family. HER2 activation triggers a downstream signaling cascade involving the phosphoinositide 3-kinase (PI3K)/AKT and RAS/RAF/MAPK signaling pathways that lead to increased cell growth, survival, and motility.

Imatinib (a low molecular weight compound)

Imatinib is a low molecular weight compound and the first clinically available ABL kinase inhibitor approved in 2001. BCR/ABL is an ideal target for molecular targeted therapy, because it is ubiquitously expressed in chronic myelogenous leukemia (CML) cells, whereas it is absent from nonmalignant cells. In addition, the BCR/ABL fusion protein is necessary and sufficient to induce leukemia. Imatinib binds to the ATP-binding site of the BCR/ABL fusion kinase and renders it inactive. This process ultimately induces a "switching-off" of downstream signaling pathways. CML was once referred to as "an incurable disease"; however, the development of imatinib mesylate improved the 5 year survival rate to > 95%.

Targeted cancer molecular therapeutics approved to date

As of April 2019, the US Food and Drug Administration (FDA) had approved 112 molecular targeted drugs for 29 cancer types. Kinase inhibitors include multi-target type, tyrosine kinase (BCR/ABL, KIT, EGFR, HER2, ALK, JAK, and BTK) inhibitors, serine/threonine kinase (mTOR, BRAF V600E mutation, MEK, and CDK4/6) inhibitors, and PI3K inhibitors. In addition, there are epigenomic inhibitors (DNA methyltransferase inhibitors, histone deacetylase inhibitors, and isocitrate dehydrogenase 2 inhibitors), proteasome inhibitors, Hedgehog signaling inhibitors, poly(ADP-ribose) polymerase (PARP) inhibitors, and Bcl-2 inhibitors. Two drugs are chimeric antigen receptor T-cell therapeutics.

Synthetic lethality and cancer

Most molecular targeted drugs act on gain-of-function mutations of oncogenes and cannot target all cancers. Developing drugs against loss-of-function mutations of tumor suppressor genes is difficult. Therefore, the identification of molecular targets based on the synthetic lethal phenotype has recently attracted attention. Synthetic lethality is used in model organisms suitable for a reverse genetics approach, such as Drosophila and yeast. Synthetic lethality refers to a phenomenon in which
a single gene deletion does not show lethality to cells or individuals but causes multiple deletions of these genes to exhibit lethality. When the synthetic lethal phenotype is used for molecular target screening in cancer, one gene is considered the causative gene of cancer or a gene mutated frequently (Figure-1). Inhibiting a partner gene product of the synthetic lethal phenotype theoretically kills only cancer cells, and normal cells are expected to survive with fewer side effects.

Olaparib

The first molecular targeted agent identified using a synthetic lethal phenotype is a PARP inhibitor (Olaparib) that was approved by the FDA and the European Medicines Agency in 2014. In 2018, it was approved in Japan for maintenance treatment of recurrent ovarian cancer sensitive to platinum-based antineoplastic agents, and later approved for BRCA gene mutation-positive and HER2-negative inoperable or recurrent breast cancer after chemotherapy. The mechanism of action of PARP inhibitors is as follows: in normal cells, DNA single-strand breaks (nicks) occurring during proliferation are repaired by PARP. If the PARP-mediated repair is unsuccessful, the nicks change to DNA double-strand breaks (DSBs) during DNA replication (in S phase) and can be restored by homologous recombination (HR) via BRCA1/2. Thus, if a repair mechanism does not work, another one will be activated. However, this complementation mechanism does not exist in BRCA1/2 mutated cancer cells. In BRCA1/2 mutated cancer cells, DSBs that occur in S phase are mainly repaired by non-homologous end joining, which simply joins the ends. This process often results in insertion-defect (InDel) mutations at the DSB site, causing frameshifts and premature stop codons that disrupt the protein reading frame of the target gene. BRCA1/2 mutant cancers are therefore highly dependent on PARP to maintain DNA homeostasis. In the presence of PARP inhibitors, normal cells can be restored, whereas BRCA1/2 mutant cancer cells cannot undergo HR, resulting in massive DNA damage and apoptosis.

Search for novel molecular targets using synthetic lethal phenotypes

In addition to BRCA1/2, synthetic lethal interactions exist for other oncogenes/tumor suppressor genes with mutations, which enabled the identification of many synthetic lethal gene pairs.
Work from our group showed that amplification of MYCN, the causative gene of neuroblastoma, and knockdown of structural maintenance of chromosomes 2 (SMC2), a condensin complex, results in a synthetic lethal phenotype. Investigation of the underlying molecular mechanism showed that SMC2, a novel target of the transcription factor MYCN, is responsible for the transcription of a DNA damage repair factor in cooperation with MYCN. In neuroblastoma cells, in which MYCN overexpression causes DNA damage, SMC2 is also highly expressed, resulting in high levels of the DNA damage repair factor and maintenance of cell survival. Knockdown of SMC2 impairs DNA damage repair leading to cell death. Analysis of expression array data and prognostic data of neuroblastoma patients indicated that amplified MYCN is associated with high expression of condensin I complex, and the expression level of condensin I subunits correlates with prognosis in patients with MYCN-amplified neuroblastoma. In other words, the group of MYCN-amplified neuroblastoma patients with low expression of condensin I subunits had a good prognosis. This suggests that MYCN amplification and SMC2 knockdown induce a synthetic lethal phenotype in cultured cells and in patients.

**Strategies to discover clinically significant synthetic lethal interactions**

Synthetic lethal screening using RNAi libraries has been performed since the 2000s to exhaustively search for genes that induce synthetic lethal phenotypes in association with genes that cause cancer when mutated or overexpressed. In this approach, genes causing cell death only in cancer cells are identified by knocking down each gene using a genome-wide RNAi library against control cells (normal cells) and cells with cancer-specific mutations\(^9\). The development of genome editing technology enabled synthetic lethal screening with fewer false positives\(^9\). In addition to these direct screenings, candidate genes are obtained from databases (SynLethDB, http://histone.sce.ntu.edu.sg/SynLethDB/) of combinations of genes showing synthetic lethality in model organisms (yeast, nematodes, Drosophila, and mouse) with accumulated reverse genetics data\(^13\). Alternatively, candidate genes can be extracted by predicting synthetic lethal genes using a database of gene mutations for each cancer\(^{14}-^{18}\). Other projects were developed to systematically identify important genes in hundreds of human cancers using loss-of-function screening of genome-wide RNAi and CRISPR libraries (Project Achilles, https://depmap.org/portal/achilles/)\(^9\). The cancer dependency map (Dep Map, https://depmap.org/portal/depmap/)\(^9\) can be obtained by integrating data from Project Achilles and the Cancer Cell Line Encyclopedia project\(^20\), and drug sensitivity data of pooled or single cell lines (Profiling Relative Inhibition Simultaneously in Mixtures\(^21\) and the Cancer Therapeutics Response Portal\(^22\)).

**Conclusions**

Although synthetic lethality is a simple idea, it had a considerable impact on the identification of molecular targets in cancer. However, most targets identified to date were verified at the basic research level, and their clinical relevance remains to be established. It should be noted that even if synthetic lethality is confirmed at the cellular level, it may not be the case in clinical trials, as in the case of EWS1-FLI1-positive Ewing sarcoma\(^23\).

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