Improving cancer therapy by combining cell biological, physical, and molecular targeting strategies

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Successful cancer therapy depends on selective killing of tumor cells while sparing normal cells. Selectivity can be achieved through treatment strategies that target tumor cells. A recent report from the Li laboratory (1) describes an elegant strategy to selectively kill tumor cells by combining several targeting strategies based on cell biological, physical, and molecular (genetic) properties of tumor and normal cells that enhances tumor cell killing *in vitro* and in an *in vivo* tumor xenograft model. The idea of using a multiplex targeting approach is reminiscent of strategies in which several antibiotics are used to treat bacterial infections while minimizing the chance that rare antibiotic-resistant mutants will arise within a population.

For decades, traditional cancer therapies exploited the difference in growth rate between most normal cells and tumor cells. The rapid growth of tumor cells depends on efficient (and more-or-less accurate) DNA replication and segregation of chromosomes to daughter cells. Many traditional chemotherapeutics prevent DNA replication directly or indirectly. For example, DNA damaging agents (e.g., cisplatin) create lesions that block replicative polymerases (2); base analogs and other agents (e.g., 5-fluorouracil and hydroxyurea) produce toxic metabolites that incorporate into DNA, and/or reduce nucleotide pools which starves cancer cells of essential DNA building blocks (3,4); and agents that directly inhibit DNA polymerases (5). Topoisomerase inhibitors such as camptothecin and etoposide have anti-cancer properties because they inhibit DNA synthesis and/or chromosome segregation (6,7). The cytotoxic effects of ionizing radiation (IR) used in radiotherapy reflect induced DNA damage, including base damage, single-strand breaks, and double-strand breaks (DSBs).

DNA lesions cause serious problems in S phase because nearly all DNA lesions block replicative polymerases, causing replication fork stalling or fork collapse to DSBs, which are particularly cytotoxic. DNA lesions are subject to repair in both normal and tumor cells, and DNA repair systems play a major role in promoting cell survival after genotoxic exposure. Rapidly dividing tumor cells have less time to repair damage before S phase initiates or continues, or before chromosomes segregate in M phase, accounting for the enhanced sensitivity of tumor cells to these agents compared to slow-growing/quiescent normal cells. When cells suffer sufficient damage, they activate cell cycle checkpoints triggering cell cycle arrest, DNA repair, or apoptosis. Checkpoint responses are frequently defective in tumor cells (8), and inappropriate progression through the cell cycle in the presence of DNA damage enhances genome instability and cell death. Thus, although traditional chemotherapeutics and IR interfere with DNA replication and chromosome segregation in both normal and tumor cells, tumor cells are often more sensitive to these agents because of their faster growth rate, a cell biological property that distinguishes tumor cells from most normal cells. The selective killing of tumor cells by these agents therefore represents a type of *cell biological targeting*.

The earliest targeted cancer therapy was surgery, which is a type of *physical targeting*. Because ionizing radiation beams can be tightly focused, high doses can be delivered to the tumor volume while minimizing the dose to nearby normal tissues. In this way, radiotherapy is another type of physical targeting. The precision with which radiotherapeutic beams can be targeted to tumors depends on the type of IR used. The most common form of radiotherapy employs high

energy X-ray (photon) beams which pass through the body and deposit energy in tissues along the entire beam path. To focus more energy to the tumor volume, modern radiotherapy employs multiple beams directed toward the tumor from different angles, termed intensity modulated radiotherapy (IMRT) (9). This maximizes the dose to the tumor while minimizing the dose to normal tissue. In particle radiotherapy (using protons or carbon ions), high energy particles deposit most of their energy when they slow and stop at the end of their path in a sharp zone termed the Bragg peak. The depth that particles travel into tissue is controlled by the initial energy of the beam. Particle radiotherapy minimizes doses to normal tissues along the entrance path, and normal tissue beyond the tumor receives little or no dose (10). Thus, particle radiotherapy is a highly effective means of physical tumor targeting.

In therapies involving genotoxic chemotherapeutics or IR, doses delivered to tumors are typically limited by doses that can be tolerated by the normal tissues. These limitations have driven the next generation of targeted cancer therapies based on identifying and exploiting specific weaknesses in tumor cells at the molecular level. There are now many examples of cancer therapies based on *molecular (genetic) targeting*. For example, certain tumors grow rapidly because cell growth signaling pathways are dysregulated, and small molecule inhibitors of protein kinases that regulate growth often have anticancer activity. One striking example is Imatinib (Gleevec), a tyrosine kinase inhibitor that is highly effective against chronic myelogenous leukemia that arises when the constitutively active BCR-ABL fusion protein is produced by chromosome translocation. Imatinib may also target the platelet-derived growth factor receptor and c-kit, and it is currently being tested against soft tissue sarcomas and gastrointestinal stromal tumors.

Although the clinical success of molecular targeting approaches is very exciting, most solid tumors are highly heterogeneous: they harbor thousands of mutations and mutation load varies dramatically among "like" tumors and even in different regions of a tumor (11,12). Thus, disrupting a single molecular target may not be generally effective with solid tumors, leading to the idea that effective treatments for solid tumors will be those that disrupt "broader biological pathways" critical for tumor growth and response to treatment (13), such as DNA repair, DNA replication, cell cycle checkpoint, and cell death pathways. Because these types of pathways are critical for cell function, pathway redundancy is common. In some cases, tumorigenesis initiates when a particular pathway

is defective; the tumor then becomes dependent on a redundant pathway. Key examples of this are breast cancers that harbor defects in the DSB repair proteins BRCA1 or BRCA2. These tumors are dependent on the PARP1 repair pathway and hence are sensitive to PARP1 inhibition. This type of molecular targeting is based on the classical genetic concept of synthetic lethality (14).

Despite the many advances in targeted cancer therapy, significant challenges remain because tumors often show intrinsic resistance to specific therapies, or they may develop resistance during treatment. Resistance to therapy can reflect upregulation of DNA repair or membrane pumps that expel drugs, or defective cell death pathways. In certain tumors hypoxic regions are specifically radioresistant. Moreover, genome instability is a hallmark of most tumors that can drive rapid tumor evolution leading to resistance to targeted therapies (15). In response to these challenges, the Li laboratory devised a strategy that combines several targeted approaches to kill tumor cells with a high degree of selectivity (1). One component of the system was an oncolytic adenovirus with a hTERT promoter-driven E1a gene, which provides conditional virus replication in telomerase-positive cells (16). Since telomerase is expressed in >85% of tumors but rarely in normal tissues, this virus is potentially useful for targeting a wide range of tumor types. The second component was a replication-defective adenovirus that expresses an shRNA directed against DNA-PKcs, which has major roles in the primary DSB repair pathway, non-homologous end-joining (NHEJ). When the hTERT-E1a and sh-DNA-PKcs adenoviruses are co-injected into a tumor, the former acts as a helper virus to allow replication of the latter, but principally within telomerase-positive tumor cells. Thus, DNA-PKcs is downregulated in the tumor cells. This downregulation of NHEJ is a second type of molecular targeting that sensitizes infected tumor cells to DSB damage. The tumors are then subjected to physical targeting using a focused IR beam, which induces DSBs in the sensitized tumor cells. Finally, because tumor cells grow rapidly, the IR-induced DNA damage represents a cell biological (growth rate-dependent) targeting approach. Because DNA-PKcs downregulation markedly sensitizes cells to DSB damage, complete tumor killing is possible with relatively low doses of IR and this reduces the damage to normal tissue. In addition, because normal tissue is not infected with the adenoviruses, these cells retain full DSB repair capacity and therefore can easily tolerate the low IR dose that they receive.

In summary, combinations of cell biological, physical,

and molecular targeting approaches represent a general framework for developing more effective cancer therapies. Because there are many types of targeted therapies, there are a vast number of combinations that can be explored. The success of specific combinations of targeted therapies will likely depend on the specificity and efficacy of each individual targeted therapy, as well as potential synergisms that may result with certain combinations.

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