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Matching targets for selective cancer therapy

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With the ever-increasing number of potential targets and advances in the development of target-selective therapeutics, choosing the right target for drug discovery and development becomes imperative. We might wish to find a crucial cancer-specific target but, with a few exceptions, no such target exists for most cancers [1,2]. If a target (e.g. kinase, transcription factor, mitochondria) is vital for a cancer cell, then it may well be vital for a normal cell. Alternatively, we may search for improved inhibitors of previously validated targets, developing new agents for the same target. But if one drug fails in the clinic for efficacy reasons, similar agents may fail too [3] unless, perhaps, they are considered for a different use [4], such as the novel anticancer strategy that will be presented here.

Let us compare the incomparable: anticancer and antibacterial drugs. The former should discriminate between cancerous and normal human cells, whereas the latter should distinguish between a prokaryotic bacterium and a human cell. There are enormous differences between bacterial and human cells; for example, the bacterial wall is absent in human cells and this target is specific and vital for bacteria. Penicillin, which targets the bacterial cell wall, is produced by fungi (eukaryotes like us) to kill bacteria without killing themselves (or human cells for that matter). In the pre-antibiotic era, there were many highly effective antiseptics available that killed bacteria (iodine, mercury, etc), but they also killed human cells. Similarly, for cancer therapy, an ideal target should be (a) essential (e.g. necessary for proliferation and/or survival) and (b) cancer-specific. The short-list of such targets includes Bcr-Abl, which is both specific and essential for chronic myelocytic leukemia [1,2]. A few other targets such as FLT in acute myeloid leukemia are emerging [2].

In search of solutions

The fundamental question is how inhibition of a non-specific target can cause a preferential anticancer effect. As suggested in the 'synthetic' lethality model, mutations in one signaling pathway may render a cancer cell vulnerable to drugs that target a parallel pathway [5,6]. For example, HSP90-active drugs, which inhibit a key cellular protein, may nevertheless preferentially kill cancer cells with mutated HSP90-client oncoproteins [7]. Similarly, DNA-damaging drugs can be preferentially toxic to cells lacking cell cycle checkpoints [8]. Finally, one drug binding several defined targets may, in theory, have selective effects towards a particular cell [9].

What do these diverse strategies have in common? There are at least two targets involved. This may not always be obvious, particularly when a second target is affected not by the drug but by a natural mutation (e.g. loss of a cell cycle checkpoint). Moreover, the second drug may affect a protective target in normal cells [10,11]. Thus, it can be stated that to achieve a selective anticancer effect, a therapeutic modality must hit not one but at least two appropriate targets. At least one of the targets would need to be dispensable for cytotoxic effect but either unavailable to, or alternatively present selectively in, a cancer cell.

The rationale for hitting more than one target

An ideal target should be (a) specific and (b) essential. Unlike bacteria, most cancer cells do not possess such targets. It is possible, however, to focus on one cancer-specific target and one essential target. Human cells contain many essential targets, such as microtubules. Microtubule inhibitors such as paclitaxel (PTX) block microtubule function in mitosis and hence kill dividing cells [12,13]. Microtubules are not, however, a cancer-specific target. PTX causes mitotic arrest and will kill both cancerous and normal cells (Figure 1a). Alternatively, loss of p53 is a cancer-specific trait (in 50% of human cancers). By inducing wt p53, low sub-lethal doses of doxorubicin (DOX) will cause G2 arrest in normal cells (Figure 1b). Therapeutic agents that induce p53 can not, however, affect cancer cells that lack p53. In other words, a 'lost' p53 is not an essential target at all. By hitting both targets, p53 and microtubules, selective cytotoxicity can be achieved in cancer cells [14]. In addition, inhibition of the Chk1 pathway by DOX can arrest certain p53-deficient cancer cells (Figure 1c). Pharmacological inhibition of Chk1 by UCN-01 solves this problem (Figure 2).

Selectivity versus synergy

It is generally assumed that the goal of combining drugs for the treatment of cancer is to achieve synergistic effects.

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This goal is justified only when the synergy is selective in cancer cells [11]. In contrast, when a synergistic combination kills all cells and living creatures, then such a combination may kill both patient and tumor. Actually, it does not matter whether drug combination is synergistic, additive or antagonistic. What is important is that drug combination should be more toxic towards the cancer cell compared to normal cells (selective synergy). For example, Hsp90 inhibitors and the topoisomerase II poison, DOX, are synergistic to cells expressing the Bcr-Abl oncogene. In contrast, they are antagonistic to parental cells [7]. UCN-01 and phorbol ester are synergistic in leukemia cells [15] even though they (inhibitor and activator of kinases, respectively) are expectedly antagonistic in some other cells. It will be important to establish their lack of synergism (one would hope that they would be antagonistic) in crucial normal tissues, such as in bone marrow.

Thus, one approach to making tumour-selective therapies is to use drug combinations that are antagonistic in normal but not in cancer cells. In other words, the goal will be to selectively protect normal cells from non-selective cytotoxic drugs. Let us now consider the triple combination of DOX-PTX-UCN-01 (Figure 2), which was suggested for the therapy of p53-deficient tumors [14]. Only one drug, namely PTX, is intended to kill cells. The two other drugs to be used, DOX and UCN-01, are included solely for the provision of selectivity [14]. Specifically, pretreatment with DOX antagonizes PTX, whereas UCN-01 abolishes this antagonism in p53-deficient cells only. Therefore, DOX and UCN-01 should be used in low non-toxic doses and in sequence (Figure 2b) rather than in parallel.

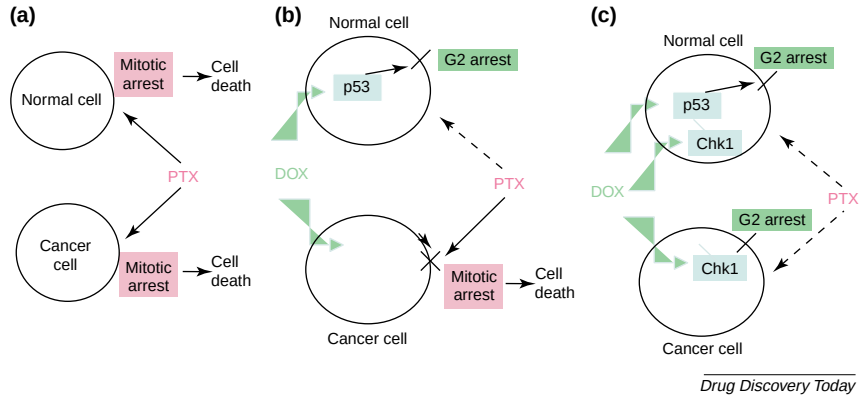

Figure 1. (a) Microtubules are a vital (essential for live of dividing cells) but non-specific target. Paclitaxel (PTX), which targets non-specific target (microtubules), inhibits mitotic spindle formation causing mitotic arrest and cell death of both normal and cancer cells. **(b)** Matching two targets: microtubules and wt p53. p53-inducing agents (e.g. low doses of doxorubicin, DOX) cause G2 arrest in cells with wt p53, without causing arrest of cancer cells lacking p53 [24]. Paclitaxel (PTX), which kills dividing cells in mitosis, then selectively kills cells lacking p53. **(c)** The strategy reveals a third matching target. In certain cancer cell lines with mutant p53, doxorubicin (DOX) induces G2 arrest via Chk1 [14]. Then both normal (wt p53) and cancer (mt p53) cells are arrested in G2 and protected from paclitaxel (PTX). This undesirable scenario can be avoided by targeting Chk1, which becomes an obvious matching target [14]. It is then necessary to include a third drug that inhibits Chk1 (see Figure 2).

Choosing targets and drug analogs

To enhance expedition, combination therapies can be designed using currently available drugs [16]. This may be rather like using a table knife instead of a surgical scalpel for brain surgery, because many of the available drugs are not wholly selective for their defined targets. We can predict, however, that once prototype combinations are suggested, new generations of more highly targeted therapies and related analogs are likely to evolve. Consider again the DOX-PTX-UCN-01 combination for selective killing of p53-deficient cancer cells. Each of these drugs has multiple, concentration-dependent mechanisms of action. To complicate matters further, the outcome is sequence dependent. Knowing the exact role of each agent in drug combination can allow the design of improved analogs or novel drugs for each target. Thus:

(a) The role of PTX is to inhibit microtubule function and kill cells that enter mitosis (Figure 2a). Therefore, other microtubule-active drugs may substitute for PTX. Furthermore, future drugs should perhaps target mitotic checkpoint proteins rather than microtubules. Using such direct mitotic inhibitors, we may avoid neurological side effects of microtubule-active drugs, which result from microtubule dysfunction in non-dividing cells.

(b) DOX arrests cells in G2 by inducing p53 (Figure 2a). DOX should be used at low doses prior to PTX, allowing protective G2 arrest (Figure 2b). Of course, DOX was not intentionally developed for the protection of cells, but rather to kill cancerous cells. It damages DNA, has a narrow cytostatic to cytotoxic therapeutic window, and is cardio-toxic. Agents that directly induce wild-type p53 [17] may be considered instead of DOX.

(c) Finally, one function of UCN-01 is to inhibit the cell cycle checkpoint kinase, Chk1 (Figure 2a). Because UCN-01 is also known to several other kinases, including protein kinases C and PDK1, more selective Chk1 inhibitors should be

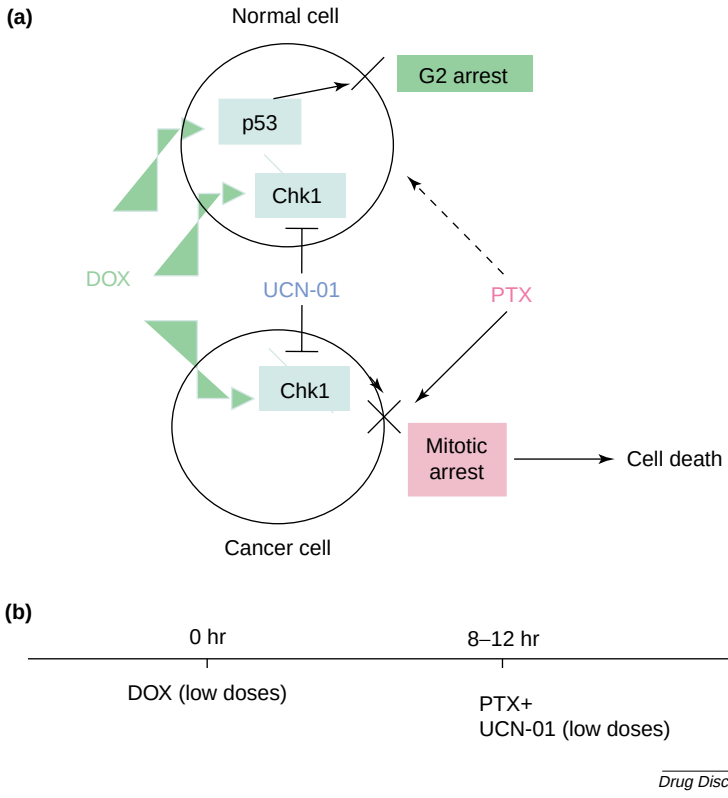

Figure 2. Matching targets and a rational combination of drugs. **(a)** Three matching targets. Low doses of doxorubicin (DOX) arrest cells in G2 via both wt p53 and Chk1. By inhibiting Chk1, UCN-01 overrides G2 arrest only in p53-deficient cancer cells (normal cells are still arrested via wt p53), thus propelling p53-deficient cells from G2 to mitosis. Once in mitosis, p53-deficient cancer cells are killed by paclitaxel (PTX) [14]. **(b)** A time-dose schedule for therapy of p53-deficient tumors. To sustain G2 arrest, DOX (low doses) should be administered 8–12 h before PTX. UCN-01 should be administered simultaneously with PTX (not with DOX). First, UCN-01 will release cancer cells from G2 into mitosis at the time when PTX is administrated. Second, UCN-01 will not antagonize DOX-induced p53 in normal cells, because UCN-01 is added after the stabilization of p53 occurs.

substituted in place of UCN-01 in the proposed drug combination. Thus, the precise purpose of each drug defines the targets and desirable properties of new drug analogs.

Discovery of 'drugs as tools'

The strategy of aiming at more than one target is likely to intensify drug discovery and development. It would dictate that one would need to develop not one but two or more drugs. Drugs are merely tools. However, in order, to kill cancer cells selectively, we will need a large arsenal of tools and a great understanding of the effects of various combinations.

For example, inhibitors of apoptosis (which are under development for the treatment of stroke or septic shock) seemingly have no application for cancer therapy. In contrast, the application of apoptosis-inducing agents has clearly been indicated in cancer therapy, especially in resistant cancers [18-22]. Unfortunately, 'it remains to be determined to what extent toxicity to normal cells will limit application of apoptosis-based therapies for cancer treatment' [20,21]. Although 'new therapeutic approaches based on drug targets in apoptotic pathways will improve the treatment of cancer patients, tumour specificity is the major issue to be resolved' [22].

I suggest that the 'matching target approach' will resolve the problem of specificity. Given that caspases may be unavailable in drug-resistant cancer cells, certain caspase inhibitors could be used to selectively inhibit apoptosis in normal cells [23]. Thus, the combination of caspase inhibitors and pro-apoptotic drugs may match genetic lesions in cancers. Then caspase inhibitors will complement apoptosis-based therapies to achieve selectivity in killing cancer cells.

Conclusion

Drug discovery and drug administration are two independent processes. Useful molecular therapeutics can be either combined for selectivity or administered singly for non-selective toxicity. Using drugs as tools to affect paired targets, we may achieve selective anticancer effects, even though no single target is available for a 'magic bullet'.

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Probiotics and prebiotics: why should the medical community pay attention?

In a recent issue of *Drug Discovery Today*, Tuohy and colleagues [1] presented a review article on the use of probiotics and prebiotics for improved gut health. Such a review raises two questions. First, why is there such a topic in a journal dedicated to 'drug discovery'? Second, is it justified to inform the medical community about scientific data on the effects of probiotics and prebiotics? Probiotics and prebiotics are classified as 'functional food ingredients' or 'food ingredients for which it can be scientifically demonstrated that they beneficially affect functions in the body relevant to well being and health or to the reduction of risk of disease' [2]. Thus they belong to nutrition not to pharmacology; they are foods, not drugs.

However, publishing the review was justified. Indeed, the primary target of probiotics and prebiotics is intestinal microflora – the large population of bacteria that colonize the large bowel and play a key role in human well being and health. The importance of the colonic microflora, particularly its composition, has largely been underestimated in medicine mainly because quantitative analysis was difficult owing to methodological problems associated with identifying, culturing and counting anaerobes. However, molecular approaches are now available that overcome these limitations and more data are now accumulating that demonstrate the key roles of some populations of bacteria, the so-called 'health promoting' bacteria, in well being and health [3]. In addition, the medical community has shown an increased interest for these products.

The review by Tuohy and colleagues is an excellent update of the data presently available. The existence of probiotics has been known for more than a century and data already exist that show beneficial effects in many medical conditions such as diarrhoea, intestinal infections and intestinal inflammation. The concept of prebiotics is more recent [4] but convincing experimental data do exist and human studies are ongoing to test different hypotheses in medically relevant situations and these show great promise. In addition, the synbiotic approach of combining both probiotics and prebiotics is attracting more and more interest.

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