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# Anticancer drug discovery in the future: an evolutionary perspective

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**Identification of agents that are pharmacologically active against human cancer has depended largely on the screening of natural products and their analogs. Many anticancer drugs have been discovered fortuitously through random investigation of organisms; indeed, serendipity remains important in anticancer drug discovery. Although it is broadly accepted that cancers comprise an evolutionary microcosm, this idea has not been advanced to understand and control carcinogenic progression. Here, we address anticancer drug discovery from an evolutionary perspective and present a series of case studies that demonstrate that the rate of anticancer drug discovery can be increased greatly by targeted screening of natural compounds from ancient species.**

## Introduction

Within the sphere of cancer chemotherapy, many commercialized drugs have been obtained by the synthesis of new compounds, from natural sources or by structural modification of natural products. Synthetic compounds, such as alkylating agents and antimetabolites, used to be the only choice for cancer chemotherapy [1]. Most of these drugs, however, injure rapidly dividing normal cells and, therefore, have substantial side effects when administered to patients. In the late 1980s, scientists began searching for selective anticancer agents that lacked the side effects associated with conventional chemotherapeutic drugs and could target 'cancer-specific' molecules to eliminate cancer cells while sparing normal cells [2,3]. Target-based anticancer agents can be classified into two categories: recombinant proteins/antibodies and low-molecular-weight compounds. Intensive research on low-molecular-weight compounds that target cell cycle regulatory proteins has led to the identification of many candidate compounds that are able to arrest proliferation and induce apoptosis in neoplastic cells. Some of these drugs – including imatinib (Gleevec, also known as STI–571), Gefitinib (Iressa, also known as ZD1839), Erlotinib (marketed as Tarceva), Bortezomib (Velcade) and tamoxifen – are approved for clinical use. Moreover, monoclonal antibodies have provided a distinct approach to the treatment of cancer [4]. Several types of antibodies with diverse pharmacological efficacy have been marketed, and many more are currently in clinical trials. Decades of research have made it clear, however, that the path to success for targeted molecules is no less arduous than the trials for cytotoxic drugs [1,3,5] because our knowledge about the total physiological functions of target molecules is limited and drugs hardly ever target just the desired molecule [6]. Consequently, some approved drugs are now being abandoned for their unexpectedly low-response rates or unforeseeable adverse effects.

An alternative source of anticancer drugs is natural products, which frequently seem to be more effective and/or less toxic. In view of the enormous biodiversity of the planet (microorganisms, plants, animals and marine organisms), a promising future for natural products seems likely; indeed, far more likely than for compounds achievable by synthesis. In 1960, the National Cancer Institute, in collaboration with the United States Department of Agriculture, initiated a large-scale screening program for antitumor agents derived from plants. During the past 50 years, more than 100 000 compounds have been screened but only seven plant-derived anticancer drugs have received Food and Drug Administration (FDA) approval for clinical application (Figure 1). In addition to terrestrial plants, marine organisms are surprising us with novel chemical structures or unusual skeletons

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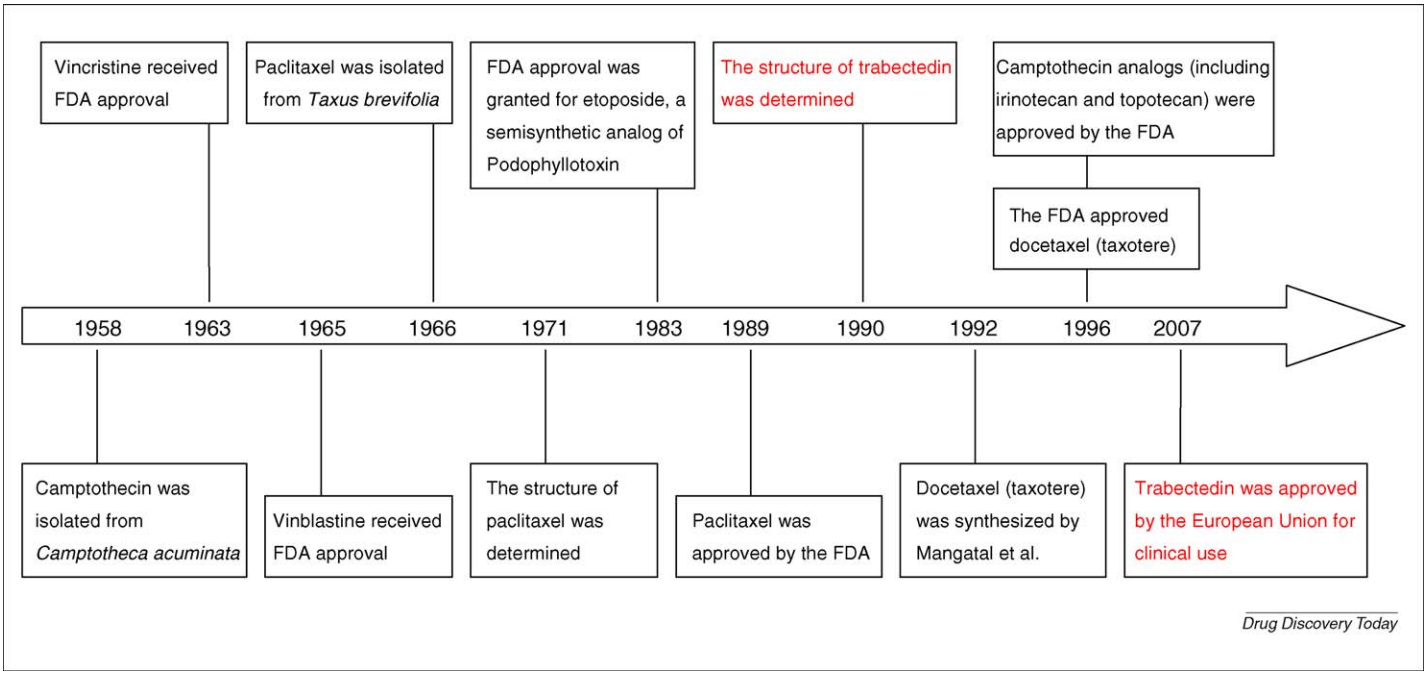


**FIGURE 1**

Timeline of natural-compound-based anticancer drug development; only those drugs with approval for clinical used are indicated.

[7,8]. Although only one marine natural product – trabectedin (yondelis/ecteinascidin-743/ET-743), which is extracted from a tropical sea-squirt – has been approved by the European Union (October 2007) for the treatment of soft-tissue sarcoma (Figure 1), several other candidate marine natural products are in different phases of clinical study for the treatment of different types of cancer [8].

To develop drugs from either marine or terrestrial sources, at least two severe problems have to be overcome. The first problem is that procurement or manufacture of quantities of rare compounds to ensure a sustainable supply might cause a bottleneck [8]. Semisynthetic procedures can sometimes circumvent these problems. This often involves extracting biosynthetic intermediates from the natural source and converting them to the final product by conventional synthesis. This approach offers two advantages: first, the intermediate might be more easily extracted in higher yield than the final product itself, and second, it might allow the possibility of synthesizing analogues of the final product with greater pharmacological activity and fewer side effects. In addition to semisynthetic procedures, the metabolic engineering of microorganisms, achieved by establishing new metabolic pathways leading to the product formation, and enforcing or removing the existing metabolic pathways toward enhanced product formation present alternative strategies for natural anticancer drug development [9]. The second problem is how one can best exploit novel anticancer agents that come from nature.

'Classical' anticancer drug development relies on, and begins with, iterative cycles of random collections of dried plant tissues for testing in proprietary bioassays followed by selection or screening for desirable compounds and, thus, serendipity is still an important route of discovery [10]. Although this classical approach for anticancer drug discovery has been somewhat successful, it is inherently slow and laborious, making the process expensive. Here, we propose a drug discovery strategy that integrates the molecular biology of cancer, evolutionary biology, cell biology and paleontology. We propose that ancient species are excellent sources of anticancer drugs; that anticancer drugs isolated from ancient species might have selective toxicity against cancer cells; and that in comparison with plants, animals are more useful organisms for the discovery of anticancer drugs. The integration of these fields might potentially advance the rate of anticancer drug discovery compared with traditional approaches.

## All plant-based anticancer drugs approved by the FDA for clinical use have been isolated from ancient species

To date, four plant-derived compounds, one marine original agent and their analogs have been approved for clinical use in United States and/or in Europe (Figure 1). All of the organisms are ancient species relative to *Homo sapiens*.

Of all the anticancer drugs, the undisputed star is paclitaxel. Paclitaxel was originally isolated from the bark of *Taxus brevifolia* in 1966 by Wani and Wall, and in 1971 its structure was confirmed [11] (Figure 1). In 1979, Schiff and Horwitz made the surprising discovery that paclitaxel stimulated microtubule polymerization [12]. Paclitaxel was initially approved by the FDA for treatment of advanced ovarian cancer in 1992 and subsequently endorsed for the treatment of metastatic breast cancer in 1994 [13] (Figure 1). Docetaxel, a closely related semisynthetic analog of paclitaxel, was also approved by the FDA in 1996 for the treatment of anthracycline-refractory advanced breast cancer and is now also used to treat lung cancer [13] (Figure 1). Plants from the *Taxus* genus are the principal source for paclitaxel (Table 1). Fossil records of Taxaceae, the gymnosperm family, extend from the onset of the Jurassic era (from approximately $199.6 \pm 0.6$ million years (Myr) ago to $145.4 \pm 4.0$ Myr ago) [14]. Comparison of either nonsynonymous nucleotide substitutions or deduced amino acid sequences of *matK* genes led Cheng *et al.* [15] to estimate that

**TABLE 1**

**Scientific classification of organisms used for isolation of anticancer compounds**

| | *Taxol* | Camptothecin | Podophyllotoxin | VinblastineVincristine | Trabectedin |
|---|---|---|---|---|---|
| **Kingdom** | Plantae | Plantae | Plantae | Plantae | Animalia |
| **Phylum**[a] | Pinophyta | Magnoliophyta | Magnoliophyta | Magnoliophyta | Chordata |
| **Class** | Pinopsid | Magnoliopsida | Magnoliopsida | Magnoliopsida | Ascidiacea |
| **Order** | Pinales | Ranunculales | Cornales | Gentianales | Enterogona |
| **Family** | Taxaceae | Berberidaceae | Cornaceae[b] | Apocynaceae | Perophoridea |
| **Genus** | *Taxus* | *Podophyllum* | *Camptotheca* | *Vinca* | *Ecteinascidia* |

[a] For historical reasons, phylum (plural, phyla) might also be called division among plants, fungi and algae.
[b] *Camptotheca* are usually included in the family of Nyssaceae, but they are sometimes included (with the tupelos) in the family Cornaceae.

the divergence between Taxads and Cephalotaxus occurred 149–179 Myr ago. The two taxad tribes originated within 6–8 Myr of the divergence event [15].

Camptothecin was also discovered by Wall and Wani during systematic screening of natural products for anticancer drugs [16]. Two camptothecin analogs, topotecan and irinotecan, have been approved by the FDA and are currently used as chemotherapeutics [17] (Figure 1). Camptothecin binds to and stabilizes the covalent topoisomerase/DNA complex, forming a ternary complex [18]. This prevents DNA re-ligation, causing DNA damage and subsequent apoptosis. Camptothecin is mainly obtained from the happy tree (*Camptotheca*) (Table 1). The earliest *Camptotheca* fossils were discovered from the tertiary period (beginning 65 Myr ago) [19].

Etoposide phosphate (brand names: Eposin, Etopophos, Vepesid and VP-16) is used to treat malignancies such as Ewing's sarcoma, lung cancers, testicular cancers, lymphomas, non-lymphocytic leukemias and glioblastoma multiforme and is derived from podophyllotoxin. Podophyllotoxin, otherwise known as podofilox, an inhibitor of the enzyme topoisomerase II [20,21], is obtained from *Podophyllum peltatum* (mayapple) and *Podophyllum hexandrum* [22] (Table 1). *Podophyllum*, sometimes referred to as intercontinental species pairs, are considered to be evolutionary leftovers from more widely distributed genera of northern-hemisphere forests during the tertiary period [23].

The source of vinblastine and vincristine is the Madagascar periwinkle, *Catharanthus roseus* [24] (Table 1). In the late 1950s, their antimitotic/chemotherapeutic potential was discovered by two groups at Eli Lilly Research Laboratories. The related family of *Vinca* alkaloids primarily target tubulin and microtubules; at high concentrations, these alkaloids depolymerize microtubules and destroy mitotic spindles, leaving the dividing cancer cells with condensed chromosomes and blocked in mitosis [25]. At low but clinically relevant concentrations, vinblastine does not depolymerize spindle microtubules, yet it potently blocks mitosis and cells die by apoptosis. The common name, shared with the related genus *Vinca*, is periwinkle. By 53 Myr ago, periwinkle and related floating ferns had completely colonized the deciduous forests.

ET-743, a significant milestone in the development of marine-derived anticancer drugs, was extracted from the Caribbean tunicate *Ecteinascidia turbinate*. A series of alkaloids was identified by Rinerhart *et al.* [26] and Wright *et al.* [27] in 1990 (Figure 1), and the most abundant active component, ET-743, and its *N*-demethyl analogue ET-729 have similar potency. Subsequently, ET-743 was selected for further development, mainly as a result of its greater abundance in *E. turbinata*. Tunicates (Table 1) evolved in the early Cambrian period, beginning 540 Myr. Despite their simple appearance, tunicates are closely related to vertebrates.

Organisms in the aforementioned five genera are considered ancient; by contrast, members of the *Homo* genus, including modern humans and their close relatives, are estimated to be approximately 2.5 Myr old, evolving from Australopithecine ancestors with the appearance of *Homo habilis*. The oldest *Homo sapiens* fossil known is from Ethiopia and is approximately 130 000 years old [28] but probably originated considerably earlier. Complete sequencing of mitochondrial DNA from 53 individuals of differing ethnic backgrounds led researchers to conclude that the common ancestor of modern human dates to 170 000 years ago, confirming that *Homo sapiens* originated in Africa at about this time [29].

## Why do ancient species produce anticancer drugs?

Identification of anticancer compounds from ancient species is likely to be successful on the basis of several lines of evidence. Most importantly, cancers are derived from mutagenic events, progressing by multiple cycles of random mutation and selection. In 1976, Nowell initially described cancer as an evolutionary system [30], and three decades of research have broadly supported this description [31–35]. A mutant or genetic variant is broadly defined as any change in genetic material that contributes to heritable variation between cells.

Darwinian natural selection is currently accepted as the initiating and main driving force of both tumor formation and tumor progression, although genomic instability contributes to the rate of tumorigenesis [31,32] (Box 1). Most researchers, however, believe that cancer is, in essence, a genetic disease; malignancy is the result of multiple cycles of accumulated gene mutations [33–36]. Within the past several decades, a diverse array of carcinogenic chemicals has been identified [37,38]; several viruses are also carcinogenic. The commonality among these agents is the propensity to generate genetic mutations. In recent years, research has clearly shown that there is genetic variability among neoplastic cell populations, and the degree of variability has been positively correlated with progression to malignancy [39]. Cancer, therefore, results from accumulation of a series of specific mutations within a cell. Although individual cancer cells have been shown to contain >11 000 genetic variants [40], only ~80 of those variants contribute to amino acid substitutions and fewer than 15 of those variants are assumed to be responsible for driving the initiation, progression or maintenance of the tumor [41,42]. In addition to single nucleotide variations, gene duplication and rearrangement are ubiquitous in cancer genomes [43,44]. According to classical

**BOX 1**

**Two most broadly accepted evolutionary theories.**

Currently, there are two main theories about the mechanism of evolution, neo-Darwinism and neutralism [53], the major difference between which is the relative importance assigned to mutation and selection. In neo-Darwinism, natural selection is assumed to have a much more important role than mutations in the process of evolution; recombination and mutation of genetic material simply provide the mechanisms through which natural selection occurs [60]. The basis of this theory is that natural selection increases the frequency of advantageous alleles at many loci, thereby enhancing the possibility that recombination will create a single individual having a novel phenotype, especially if interactions between genes are effected.

In 1968, Kimura formally proposed the neutral theory [54] of molecular evolution and provided substantial evidence for the theory. This landmark theory claims that genetic mutations are the primary cause of evolutionary change. The theory also asserts that although mutations can be deleterious, neutral or adaptive, most of the genetic variability within species at the molecular level is selectively neutral and that these mutations are maintained in the population by the balance between mutation input and random extinction. According to Kimura, mutations are neutral when $|2Ns| < 1$ or $|s| \leq 1/2N$, where $N$ is the effective population size, $s$ is the selection coefficient for mutant heterozygotes and $2s$ is the selection coefficient for mutant homozygotes. This theory succeeded in explaining many aspects of molecular evolution, but sequence data have revealed some discrepancies with respect to neutral theory predictions at replacement sites. In 1973, Ohta [55] expanded the theory to include not only selectively neutral mutations but also deleterious mutations and, importantly, slightly deleterious mutations—those that have a sufficiently small selective effect to enable propagation in small populations but prohibit propagation in large populations. According to neutralism, in contrast to the neutralist view of molecular evolution, evolution at the phenotype level is almost exclusively adaptive and caused by Darwinian positive selection [52].

Inherited traits are controlled by genes, and the complete set of genes within an organism is called its genotype. The complete set of observable traits that comprise the morphological and behavioral aspects of an organism is called its phenotype. Phenotypic variations in a population reflect variations in individual genotypes. For this reason, Nei [52] argued that the basic process of phenotypic evolution is essentially the same as that of molecular evolution. Nei [52] contended that although most mutations do not change phenotypic character of a population and, therefore, are selectively neutral (or nearly so), mutation remains the primary force behind genotypic and phenotypic evolution. He defined this theory as neomutationism [53] and also argued that neutral mutations should be defined as mutations that do not influence the fitness of species or the individuals within the species.

cancer genetics, the majority of cancer-causing mutations occur in oncogenes, thereby enhancing gene expression or function, and/or tumor suppressor genes, thereby causing loss of gene expression of function [45,46].

Oncogenes were initially identified as genes carried by viruses that cause transformation of their target cells. The majority of viral oncogenes have cellular counterparts called proto-oncogenes that are involved in normal cell function. Many oncogenes involved in various stages of human cancer – tumor initiation, progression, angiogenesis and metastasis – have been discovered. These oncogenes encode proteins with a variety of cellular functions, ranging from transmembrane proteins to transcription factors, and elucidation of their normal cellular functions might, therefore, lead to an understanding of the types of genetic changes that are involved in tumor formation. Oncogenes are generated by mutations that render a gene constitutively active or active under conditions in which the wild-type gene is inactive. The generation of an oncogene represents a gain-of-function mutation in which a cellular proto-oncogene is inappropriately activated; in certain cases, mutation or aberrant activation in the cell results in tumor formation [47]. This can result from mutations that give rise to altered protein sequence or from constitutive gene activation, overexpression or deregulation of temporal gene expression. Therapeutic strategies targeting oncogenes have been the focus of intense research over the past several decades [48].

By contrast, mutation of tumor suppressor genes reduces the activity of the gene products. These mutations represent loss-of-function changes in genes that normally function to constrain the cell cycle or cell growth; the release of the constraint is tumorigenic [49]. In most cases, it is necessary for both copies of the gene to be inactivated. This is achieved by mutations that result in premature termination of translation, by insertions or deletions of varying sizes, or by epigenetic silencing. Oncogene and tumor suppressor gene mutations result in similar physiological changes—they drive neoplasia by increasing tumor cell numbers through stimulation of cell division or by inhibition of cell death or cell cycle arrest. These genes have been called 'gatekeepers' [34] because they directly regulate the growth and differentiation pathways of cells.

Mutations in genes that regulate genomic stability ('caretakers') and the extracellular matrix ('landscapers') also promote tumorigenesis [34]. Stability genes include those involved in mismatch repair, nucleotide-excision repair and base-excision repair that are responsible for repairing subtle mistakes made during normal DNA replication or alterations induced by exposure to mutagens. Stability genes also include those that control processes that maintain large portions of chromosomes, such as those responsible for mitotic recombination and chromosomal segregation. Stability genes keep genetic alterations to a minimum; when stability genes are inactivated, mutations arise more rapidly in other genes [46]. For this reason, mutation of stability genes can lead to genomic instability, resulting in rapid accumulation of changes in other genes that directly control cell division and death. Mutations in landscaper genes do not directly affect cell growth but rather generate an abnormal extracellular matrix environment that contributes to cellular neoplasia. The extracellular matrix, which is produced by collaboration between stromal fibroblasts and epithelial cells, comprises the cellular scaffolding and provides cell identity. Epithelial cells form intercellular junctions to generate polarized sheets, and the endothelial vasculature provides nutrients and oxygen to cells and harbors immune cells that combat pathogens and remove apoptotic cells. Most of the antitumor agents developed to date target oncogenes, tumor suppressor genes, stability genes and landscaper genes and/or their expressed products.

Somatic mutations are the primary cause of cancer development in patients of all ages and in all tissue types; this process explains most of the variation in the age of cancer incidence. Therefore,

elimination of mutations and their subsequent effects is pivotal to the prevention and cure of cancer.

Production of anticancer compounds from ancient species is also likely to be successful because these species synthesize small molecules that slow the rate of phenotype evolution and, therefore, have the potential to arrest carcinogenesis. Compared with other organisms, the phenotypes of ancient species evolve more slowly. According to all evolutionary theories, evolutionary rates can vary on the basis of the interplay between mutations and selection [50,51] (Box 1). These underlying factors are often correlated and lead to the conclusion that lower mutation rates and high selection pressure cause slower evolutionary rates.

Mutation is the driving force of evolution [52–55]. In all living organisms, DNA continually incurs damage [46,51,56]. Cells have evolved complex signaling pathways to arrest the progression of the cell cycle in the presence of DNA damage. Genomic integrity is preserved, partly, by mechanisms that ensure that cell cycle progression is delayed until DNA damage is removed. These so-called checkpoint mechanisms can lead to delays in the G1, G2 or S phase of the cell cycle. Several checkpoint components have functions related to activation of DNA repair, nucleotide metabolism, telomere maintenance or induction of cell death. Currently, these responses, in addition to other checkpoint mechanisms, are collectively referred to as 'DNA damage–response pathways' [45].

DNA mutations are minimized by repair systems that can recognize and correct the damage [46,56,57]. The extensive biochemical similarities in DNA repair processes between phylogenetically diverse model species such as *Escherichia coli*, *Saccharomyces cerevisiae* and humans [43] suggest that DNA repair is probably too invariant to influence mutational variation. When a repair system corrects DNA damage, there is no consequence. Mutations can result, however, when the repair system fails. At the cellular level, damaged DNA that is not properly repaired can lead to genomic instability, apoptosis or senescence [57,58]. Cells are able to initiate apoptosis [46,56] when the burden of genomic insult is simply too great to be effectively met by the various responses available; apoptosis eliminates damaged cells while sparing normal cells so that serious pathological consequences are avoided. Cellular senescence is a condition in which living cells can no longer proliferate. Senescence is a stress response and, therefore, differs from quiescence or terminal differentiation. Replicative senescence halts proliferation of normal human cells [58]. The exposure of cells to many DNA-damaging agents results in transcriptional upregulation of a large number of genes, the precise functions of many of which remain to be established but are likely to involve elimination of mutations.

Selection is the process by which genetic mutations that enhance reproduction become and remain more common in successive generations of a population. This process is proposed to work at the level of genes, cells, individual organisms, groups of organisms and even species [59] and might act simultaneously at multiple levels. Selection of carcinogenic mutations occurs mainly at the cellular, tissue and whole-organism levels [31]. Selection can be divided into two parts: self-selection and natural selection. Selection at the gene, cell and individual organism levels is defined as self-selection and is controlled by complex processes of temporal and spatial expression of many interacting genes. Selection at the group and species levels is defined as natural selection. The effect of natural selection on the phenotype is controlled by genotype because without gene variation, phenotypic changes cannot be transmitted to offspring [59,60].

Ancient species probably evolved more slowly owing to fewer accumulated mutations (greater DNA sequence stability) resulting from a higher incidence of apoptosis and senescence; mechanisms that have yet to be revealed might also affect evolutionary rate. Cellular apoptosis and senescence also have a substantial effect on cancer development [46,56,58]. Macromolecules, such as DNA, RNA and proteins, are responsible for these cellular processes and are, in turn, regulated by small organic molecules. These small molecules are responsible for the most fundamental chemical processes essential for life, such as those that produce cellular energy and those that transmit messages to cells and their components [61]. Because small molecules contribute to such fundamental cellular processes, it is not surprising that they are also essential for processes involved in deceleration of evolution, elimination of cancer cells and regulation of cellular responses to cancer. These small molecules, therefore, should be considered as candidates for anticancer drug development. Take, for example, the fact that paclitaxel binds to the intermediate domain of β-tubulin via hydrogen bonds and hydrophobic contacts, thereby promoting polymerization and interfering with microtubule dynamics [13]. Microtubules have important functions in cellular activities such as maintenance of shape, movement, signaling, division and mitosis. Therefore, the binding of paclitaxel to microtubules suppresses spindle microtubule dynamics, thereby inhibiting the metaphase anaphase transition, blocking mitosis and inducing apoptosis [13]. Other anticancer natural products also interfere with important cellular functions.

Anticancer molecules isolated from ancient species might lack side effects. As mentioned above, most compounds are normal cellular metabolites and function in signal transduction, therefore when new phenotypes arise as a result of mutations, these molecules induce aforementioned mechanisms such as apoptosis and causing senescence. Most of these mechanisms eliminate abnormal cells while sparing normal cells [46,56,58].

## Ancient animals and anticancer drugs

Living organisms are grouped into three general domains: Archaea, Bacteria and Eukarya. Eukaryotes are subdivided into four kingdoms: protists, fungi, plants and animals. All organisms share many biological characteristics, such as having single or multi-cellular composition, carrying out metabolism and energy transfer via ATP, and encoding hereditary information in the form of DNA [60].

Because the members of the animal kingdom have similar cellular and organismal properties, many animal-derived drugs, such as ET-743, have been developed. Other examples include blood-feeding insects, leeches and bats, which have developed effective anti-coagulants; peptides from their saliva have been isolated, and some are now in clinical trials for treatment of circulatory disorders. We propose that not only plants and microorganisms but also ancient animals should be potential sources for candidates for anticancer drugs. Anticancer drugs isolated from ancient animals might be more successful than those isolated from plants, not only because ancient species probably

harbor molecules that have contributed to their slower rate of evolution but also because all animals have much in common, such as metabolic networks, signal transport pathways and, most importantly, synthesis of small molecules in response to exposure to mutagenic agents.

## Concluding remarks

Natural products have been an important source of chemotherapeutics for the past 30 years; more than half of effective cancer drugs can be traced to natural origins. Development of naturally derived anticancer drugs, therefore, is crucial, and isolation of novel compounds has become an important part of cancer research. Most anticancer drugs have been discovered through random screening of organism collections, but our improved understanding of many of the molecular details of carcinogenesis and evolution makes it possible to develop more efficient strategies; specifically, targeting ancient species for the isolation of natural products with anticancer activity.

We thus encourage researchers to consider ancient species, particularly ancient animals, for development of potential anticancer agents. This is likely to greatly enhance the success rate of identifying novel anticancer drugs.

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