Biotechnology Advances 36 (2018) 506–520

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

# Plants as sources of natural and recombinant anti-cancer agents

J.F. Buyel[a,b,*]

[a] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstraße 6, 52074 Aachen, Germany
[b] Institute for Molecular Biotechnology, Worringerweg 1, RWTH Aachen University, 52074 Aachen, Germany

ARTICLE INFO

*Key words:*
Lectins
Molecular farming
Monoclonal antibodies
Plant secondary metabolites
Therapeutic anti-cancer vaccines

ABSTRACT

Herbal remedies were the first medicines used by humans due to the many pharmacologically active secondary metabolites produced by plants. Some of these metabolites inhibit cell division and can therefore be used for the treatment of cancer, e.g. the mitostatic drug paclitaxel (Taxol). The ability of plants to produce medicines targeting cancer has expanded due to the advent of genetic engineering, particularly in recent years because of the development of gene editing systems such as the CRISPR/Cas9 platform. These technologies allow the introduction of genetic modifications that facilitate the accumulation of native pharmaceutically-active substances, and even the production heterologous recombinant proteins, including human antibodies, lectins and vaccine candidates. Here we discuss the anti-cancer agents that are produced by plants naturally or following genetic modification, and the potential of these products to supply modern healthcare systems. Special emphasis will be put on proteinaceous anti-cancer agents, which can exhibit an improved selectivity and reduced side effects compared to small molecule-based drugs.

## 1. Introduction

### 1.1. Socio-economic relevance and causes of cancer

Despite decades of medical research, cancer remains a major challenge to healthcare systems (Yabroff et al., 2011). There was an increase from 6.2 to 8.8 million cancer-related deaths between 2003 and 2015, equivalent to approximately 13% of all deaths worldwide (Bray et al., 2012; McGuire, 2016; Stewart and Wild, 2014; WHO, 2017). The cancer-related mortality rate is higher in developing countries than industrialized countries due to socio-economic conditions that restrict access to anti-cancer therapies (Sankaranarayanan, 2014). More than 14 million new cancer cases are reported each year, and this is expected to increase by 26% over the next 35 years due to demographic changes and improved diagnostics (Pritzkuleit et al., 2010; Rottenberg et al., 2010). Cancer has a devastating physiological and psychological impact on patients and their families (Faller et al., 2013; Linden and Girgis, 2012), but on a broader level it also imposes a massive economic burden on society, with an estimated $895.2 billion in healthcare-related payments and reduced productivity in 2008 (American Cancer Society, 2010).

Cancer is not a narrowly-defined condition with a single cause, but a group of more than 100 different diseases with shared characteristics (American Cancer Society, 2015). Different cancers have different incidences in particular demographic groups, reflecting genetic, developmental and environmental factors. For example, breast cancer is much more common in women than men due to developmental differences between the sexes, whereas lung cancer is more common in men because more men are exposed to environmental triggers through employment or smoking. Even so, lung cancer is the most frequent cancer-related cause of death overall, with 1.59 million cases in 2014 (American Cancer Society, 2015; McGuire, 2016).

The common characteristic shared by all types of cancer is that a subset of cells acquires the ability to undergo rapid and uncontrolled proliferation. In most cases, this is initially associated with the formation of spatially-confined primary tumors in the affected tissue (the exception being hematological malignancies, which arise from blood cells or their progenitors). Some tumors arrest at this stage and remain benign and non-invasive, and are not classified as cancers (Silverstein et al., 2006). However, advanced tumors become progressively more

*Abbreviations:* APIs, active pharmaceutical ingredients; ADCC, antibody-dependent cellular cytotoxicity; ADCs, antibody–drug conjugates; ATPS, aqueous two-phase systems; CHO, Chinese hamster ovary; CDC, complement-dependent cytotoxicity; DoE, design-of-experiments; EBV, Epstein–Barr virus; EBA, expanded-bed adsorption; FDA, Food and Drug Administration; GMP, good manufacturing practice; HBsAg, hepatitis B soluble antigen; HBV, Hepatitis B virus; HCPs, host cell proteins; HPV, Human papillomavirus; ML1, mistletoe lectin 1; mAbs, monoclonal antibodies; NK, natural killer; R&D, research and development; PEG, polyethylene glycol; PAT, process analytical technology; QbD, quality-by-design; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; $T_h1$, T-helper; T-DNA, transfer DNA; VEGF, vascular endothelial growth factor; VFUs, vertical farming units; VLPs, virus-like particles

* Corresponding author at: Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstraße 6, 52074 Aachen, Germany.
*E-mail addresses:* johannes.buyel@rwth-aachen.de, johannes.buyel@ime.fraunhofer.de.

https://doi.org/10.1016/j.biotechadv.2018.02.002
Received 3 July 2017; Received in revised form 23 January 2018; Accepted 1 February 2018
Available online 03 February 2018
0734-9750/ 

dysregulated and malignant: the tumor cells can not only invade adjacent tissues but also detach from the primary tumor, spread through the blood and/or lymphatic system, and form secondary tumors elsewhere, a process known as metastasis (Alberts et al., 2002). Initially, six properties were proposed to define cancer: (i) self-sufficiency in growth signals, (ii) insensitivity to anti-growth signals, (iii) evasion of apoptosis, (iv) limitless replicative potential, (v) sustained angiogenesis, and (vi) potential for tissue invasion and metastasis (Hanahan and Weinberg, 2000). Four additional characteristics have been proposed more recently: (vii) dysregulated metabolism, (viii) evasion of the immune system, (ix) genome instability, and (x) induction of inflammation (Hanahan and Weinberg, 2011). However, these properties may overemphasize the cellular aspects of the disease compared to the impact on tissues (Sonnenschein and Soto, 2013).

The onset of cancer is often spontaneous and linked to abiotic risk factors such as smoking, high-energy radiation, or carcinogenic chemical substances, but genetic predispositions can increase the likelihood of disease or reduce the age of onset. Biotic risk factors include infections with certain viruses (Cummins and Tangney, 2013; Weinberg, 2006), such as Human papillomavirus (HPV) as the major cause of cervical cancer (Chen et al., 2015), and presumably Epstein–Barr virus (EBV), which is linked to Burkitt's lymphoma (Brady et al., 2007), as well as some bacterial infections, e.g. *Helicobacter pylori* (Hong et al., 2012). Ultimately, all factors trigger mutations and/or epigenetic changes in the DNA structure that inactivate tumor suppressor genes such as *TP53* (Bieging et al., 2014) or activate proto-oncogenes such as *HER-2* (Chial, 2008). Some of the factors can immediately cause mutations including: (i) the induction of point mutations by alkylating agents, nucleoside analogs or intercalating chemicals, (ii) the erroneous repair of DNA double strand breaks induced predominantly by radiation, or (iii) the integration of foreign DNA, disrupting the original genetic context and causing aberrant gene expression as observed for some viruses (Akagi et al., 2014). Other factors act indirectly, e.g. by inducing chronic inflammation or infections that promote the proliferation of a subset of cells, e.g. B-lymphocytes, increasing the likelihood of uncontrolled growth as assumed for EBV in Burkitt's lymphoma.

### 1.2. Cancer therapy strategies

Due to the heterogeneous nature of cancer there is no universal treatment, but four different general approaches have evolved, which can be applied either alone or in combination (Sudhakar, 2009).

#### 1.2.1. Surgery

Surgery involves the physical removal of malignant tumor tissue, which in theory can provide a complete cure with a single treatment. However, a small number of residual cancer cells may remain at the excision site, eventually leading to the formation of a new tumor, or pre-malignant cells can be activated. This can be prevented by expanding the excision to adjacent healthy tissue although this can have severe side effects, especially in the case of brain tumors, and does not necessarily increase the survival rate (Hernandez et al., 2009; Kubota, 2011). If metastasis has occurred, it can be difficult to locate and remove all small secondary tumors, which is why surgery is often combined with chemotherapy or radiotherapy to increase the likelihood of a cure (Salama and Chmura, 2014).

#### 1.2.2. Radiotherapy

In radiotherapy, tumor tissue is deliberately exposed to X-rays or gamma rays in order to induce DNA damage that cannot be repaired in rapidly-dividing cancer cells, causing them to arrest during DNA replication and undergo apoptosis. The accuracy of radiotherapy is limited because ensuring that tumor cells receive a lethal dose necessarily exposes surrounding healthy cells to lower but still harmful doses of radiation. DNA repair is more efficient in healthy cells but nevertheless they are often damaged or even killed (Bernstein and Bernstein, 2015; Gajecka et al., 2005). The side effects of radiotherapy can therefore include the temporary depletion of hematopoietic precursor cells, and in the longer term may even induce mutations that lead to other forms of cancer (Berrington de Gonzalez et al., 2011; Mauch et al., 1995). The effectiveness of radiotherapy requires a sufficient supply of oxygen because the limited direct effect of radiation on DNA is boosted by the generation of mutagenic free radicals when the radiation interacts with oxygen molecules. However, hypoxia often occurs in large solid tumors, which means that radiotherapy is less effective in this context (Harrison et al., 2002).

#### 1.2.3. Chemotherapy

Chemotherapy refers to the treatment of cancer with drugs containing low molecular mass active pharmaceutical ingredients (APIs), such as the abovementioned plant-derived mitotic inhibitor paclitaxel (Chabner and Roberts Jr, 2005). This approach is advantageous because the APIs can typically circulate relatively freely within body fluids, allowing them to reach tumor sites following injection into the blood, and the drugs can also kill remote and circulating cancer cells as well as small, undetectable secondary tumors even if their precise location is unknown (Polireddy and Chen, 2016). Chemotherapy can also be combined with radiotherapy to increase therapeutic efficacy (Shahid, 2016). However, the efficacy of chemotherapy largely depends on the ability of the drug to penetrate tumor tissue, which is often impaired in large, solid tumors due to the limited vascularization (Minchinton and Tannock, 2006).

The first generation of chemotherapeutics were developed to disrupt the metabolic and/or mitotic activity of rapidly dividing cells, whereas the second generation instead targeted signaling components, such as protein kinases and growth factor receptors (Chabner and Roberts, 2005). For example, paclitaxel is a first-generation drug that disrupts mitosis by preventing tubulin depolymerization, whereas gefitinib is a second-generation drug that inhibits signaling via the epidermal growth factor receptor (Chabner and Roberts, 2005; Wani and Horwitz, 2014).

Some APIs used as anti-cancer agents have a simple molecular structure facilitating their production by chemical synthesis (Neidle and Thurston, 2005), but most of them are complex molecules that must be produced using biotechnology (Baldi et al., 2008; Howat et al., 2014). Paclitaxel falls into the latter category. This compound was originally isolated from the bark of the Pacific yew tree (*Taxus brevifolia*) (Wani and Horwitz, 2014), but is now produced in transgenic plant cell suspension cultures at the 75,000-L scale (Zhong, 2002). Many chemotherapeutic agents are selective rather than specific in terms of the cells they affect, i.e. some act on all rapidly dividing cells including those found in tumors but also those in healthy tissues, such as hair follicle cells and B-lymphocytes. As a consequence, typical side effects of chemotherapy include hair loss and a compromised immune system (Sfikakis et al., 2005; Trueb, 2010).

#### 1.2.4. Immunotherapy

Immunotherapy harnesses the immune system in the fight against cancer, and is the most selective treatment approach and therefore the treatment associated with the least severe side effects (Caspi, 2008; Schuster et al., 2006). Chemotherapy can be combined with immunotherapy (Bang et al., 2010). The APIs used in immunotherapy are often proteins or peptides, including prophylactic or therapeutic vaccines to prevent the onset of cancer before or after exposure to biotic risk factors, as seen with the vaccines against HPV to prevent cervical cancer (De Vincenzo et al., 2013; Poljak, 2012). Other strategies focus on the introduction of cytokines to manipulate the immune response, or use antibody therapy to target cancer cells in the same way that antibodies normally target pathogens (Schuster et al., 2006).

In the latter case, monoclonal antibodies (mAbs) are directed against cancer-specific cell surface structures. Such structures can include receptors and other surface proteins that are overexpressed in