Journal of Bioscience and Bioengineering
VOL. 111 No. 5, 501 – 511, 2011

www.elsevier.com/locate/jbiosc

REVIEW

Development of natural anti-tumor drugs by microorganisms

Chia-Che Chang,[1,2,†] Wei-Chuan Chen,[3,†] Tsing-Fen Ho,[4] Ho-Shing Wu,[5] and Yu-Hong Wei[3,*]

Institute of Biomedical Sciences, National Chung Hsing University, Taichung 40227, Taiwan,[1] Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan,[2] Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Chung-Li, Taoyuan 320, Taiwan,[3] Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung 40601, Taiwan,[4] and Department of Chemical Engineering and Material Science, Yuan Ze University, Chung-Li, Taoyuan 320, Taiwan[5]

Received 12 November 2010; accepted 30 December 2010
Available online 28 January 2011

Discoveries of tumor-resistant pharmacological drugs have mainly resulted from screening of natural products and their analogs. Some are also discovered incidentally when studying organisms. The great biodiversity of microorganisms raises the possibility of producing secondary metabolites (e.g., mevastatin, lovastatin, epothilone, salinosporamide A) to cope with adverse environments. Recently, natural plant pigments with anti-tumor activities such as β-carotene, lycopene, curcumin and anthocyanins have been proposed. However, many plants have a long life cycle. Therefore, pigments from microorganisms represent another option for the development of novel anti-tumor drugs. Prodigiosin (PG) is a natural red pigment produced by microorganisms, i.e., *Serratia marcescens* and other gram-negative bacteria. The anti-tumor potential of PG has been widely demonstrated. The families of PG (PGs), which share a common pyrrolylpyrromethene (PPM) skeleton, are produced by various bacteria. PGs are bioactive pigments and are known to exert immunosuppressive properties, *in vitro* apoptotic effects, and *in vivo* anti-tumor activities. Currently the most common strain used for producing PGs is *S. marcescens*. However, few reports have discussed PGs production. This review therefore describes the development of an anti-tumor drug, PG, that can be naturally produced by microorganisms, and evaluates the microbial production system, fermentation strategies, purification and identification processes. The application potential of PGs is also discussed.

[**Key words:** Cancer; Natural anti-tumor drugs; Prodigiosin (PG); Microbial production system; Anti-tumor activities]

Cancer is now a major cause of death in Taiwan. Cancer is characterized by unlimited growth, invasion, and metastasis of cells whereas benign tumors are self-limiting, non-invasive, and non-metastasizing (1,2). Most clinical symptoms are accompanied by weight loss, poor appetite, fatigue, unusual lumps, bleeding, pain, enlarged lymph nodes, and neurological symptoms (3,4). Common environmental causes of cancer include chemicals (e.g., tobacco), ionizing radiation (e.g., ultraviolet radiation), infection (e.g., human papillomavirus), heredity, lack of physical activity, and environmental pollutants. The unique properties of cancer cells include dysregulated proliferation, immortalitization, metastasis and angiogenesis. Tumor angiogenesis is the proliferation of blood vessels that supply nutrients and oxygen and that remove metabolic waste from tumors. Tumor angiogenesis involves the interaction of tumor cells, endothelial cells, phagocytes and their secreted factors which may promote or inhibit angiogenesis. Therefore, inhibiting tumor angiogenesis is a major goal of cancer treatment.

Clinical treatments for cancer therapy include chemotherapy, radiation therapy, surgery, immunotherapy, and other methods. In recent years, anti-tumor drugs have frequently been used for chemo-cancer therapy (5). The many commercially available anti-tumor drugs can be classified by origin as either chemical synthetic drugs (e.g., alkylating agents and antimetabolites) or natural drugs derived from organisms (e.g., Taxol, Camptothecin and Trabectedin) (5,6). Synthetic drugs are often the only option for cancer chemotherapy (5–7). However, most synthetic drugs kill not only tumor cells, but also normal cells, and most have severe side effects (8). Natural anti-tumor drugs derived from organisms have also proven effective and less toxic for cancer therapy (5,8,9). Promising natural products should be more than synthetic drugs by the huge distribution of earth. Large-scale screenings of microorganisms, plants, animals and marine organisms for anti-tumor drugs have been performed in recent decades (8).

Since the 1940s, more than 100,000 natural anti-tumor drugs have been identified. In recent decades, many natural anti-tumor drugs from animal, plant, microorganism and marine organisms have been identified and approved by Food and Drug Administration (FDA) (Table 1A) (8–19). However, most of the natural drugs listed in Table 1 have not been approved by the FDA. Some drugs such as mevastatin are produced from microorganisms and are believed to be potent inhibitors of tumor angiogenesis, (Table 1B) (8–19). However, these drugs are still not approved by the FDA. Even drugs without FDA

* Corresponding author. Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Chung-Li, Taoyuan 320, Taiwan. Tel.: +886 3 4638800; fax: +886 3 4334667.
E-mail address: yhwei@saturn.yzu.edu.tw (Y.-H. Wei).
† The first two authors contributed equally to this work.

1389-1723/$ - see front matter © 2011, The Society for Biotechnology, Japan. All rights reserved.
doi:10.1016/j.jbiosc.2010.12.026

TABLE 1. Anti-tumor drugs approved by FDA (A) and anti-tumor drugs from microorganisms without FDA approval (B).

A

Kingdom	Name	FDA approved
Animal	Alemtuzumab	Yes
Animal	Bevacizumab (avastain)	Yes
Animal	Cetuximab	Yes
Animal	Panitumumab	Yes
Animal	Tositumomab	Yes
Plant	Palictaxel nanoparticles	Yes
Plant	Vinorelbine (semi)	Yes
Microorganism	Lovastatin	Yes
Microorganism	Simvastatin (semi)	Yes

B

Strains	Anti-tumor drugs	Refs
Yeast	Beta-glucan	10
Lechevalieria aerocolonigenus	Becatecarin	11
Micromonospora sp.	Eco-4601	12
Salinispora tropica	NPI-0052	13
Chromobacterium violaceum	Romidepsin	14
Taxus cuspidata	Taxol	15
Streptomyces alanosinicus	Spicamycine	16
Streptomyces peucitius	Amrubicin hydrochloride	17
Streptomyces sp. MDG-04-17-069	Tartrolon D	18
Streptomyces chartreusis	Elsamirucini	19
Serratia marcescens	Prodigiosin	8,9

approval, microorganisms are still the most promising sources of natural anti-tumor drugs. Among all organisms on earth, microorganisms have the greatest biodiversity. Some microorganisms that can survive in abdominal environments can secrete metabolites to protect themselves. Some of these metabolites have potential medical applications. Secondary metabolites from microorganisms with potential anti-tumor activities have been discovered in recent years. Five microorganism systems (cyanophytes, marine microbes, extremophiles, microbial symbionts and plant endophytes) are now known to produce secondary metabolites against abdominal environments and for self-protection (20–25). Examples include taxol, curcumins, actinorhodin, roseophilins (26–28), and marineosins (29).

Some natural plant pigments such as β-carotene, lycopene, curcumin and anthocyanins are also believed to have anti-tumor properties. However, many plants have a long life cycle. Therefore, pigments from microorganisms are another option. Prodigiosins (PGs) with red pigment are the secondary metabolite produced from *Serratia* sp. and other unrelated microbial strains such as *Vibrio psychroerythrus, Streptomyces griseoviridis*, and *Hahella chejuensis* (26,27,30). The PG was first found in the broth of *S. marcescens* in 1902. A characterization of the chemical structure of PG in Reapoport and Holden (31) revealed a molecular weight of 320.4 amu (31). The PGs have a common pyrrolyldipyrrolylmethene (PPM) skeletal core with different alkyl substituents such as a linear carbon chain and cyclic derivatives that form PG derivatives, i.e., a family of naturally secreting pyrrole red pigments that are secondary metabolites from microorganisms (Fig. 1) (32). The PGs include the linear tripyrrole undecylprodiginine and cyclic derivatives (including butylmetacycloheptylprodigiosin, butylcycloheptylprodiginine metacyclononylprodigiosine and nonylprodigiosin). In recent years, PGs have been characterized to be antimicrobial, antimalarial, immunosuppressive and cytotoxic (33). The advantages of PGs in anti-tumor drug development include only a shorter preparation time and a different pathway for anti-tumor treatment than other natural drugs, but also the potent immunosuppressive, antifungal, antiproliferative, and proapoptotic properties of PGs (33). In contrast, caveats exist such as the high production costs and the complicated purification and

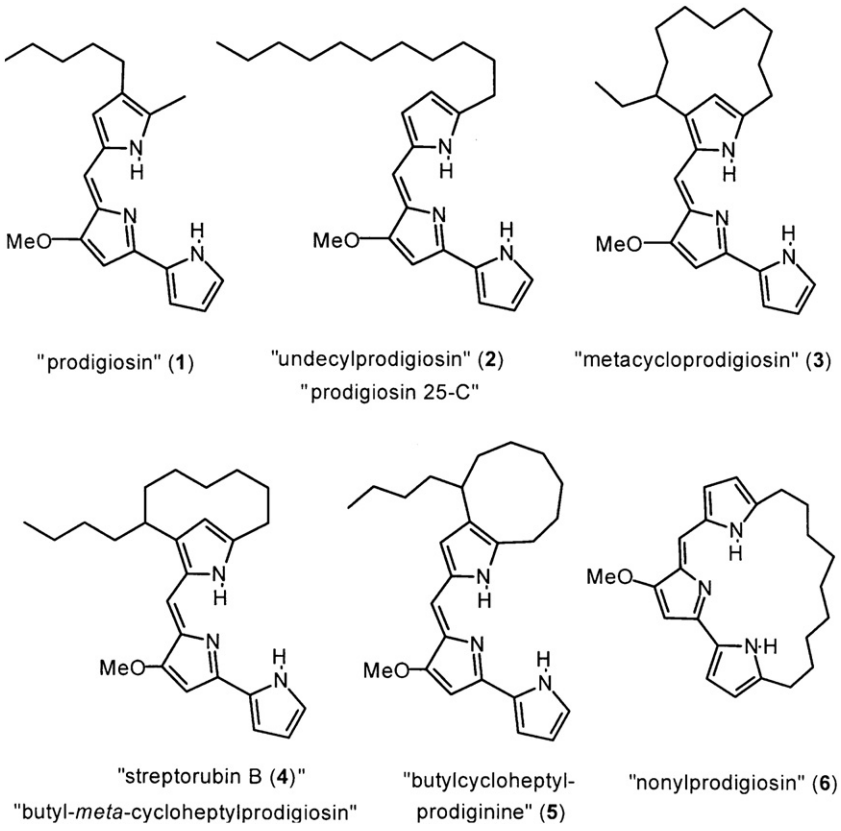

FIG. 1. Chemical structures of PGs (32).

isolation processes from the fermentation broth of different microorganisms. In this review, we will aim at exploring PG as an anti-tumor drug that is naturally produced by microorganisms, and also evaluate the microbial production system, fermentation strategies, purification and identification processes, and finally the application potential of PGs.

MICROBIAL PRODUCTION SYSTEMS OF PRODIGIOSINS

Natural compounds in PGs are known to have a broad range of toxic cellular activity (32). Extensive studies of PG synthesis in *Serratia* sp. were proposed. *S. marcescens* is the major bacterium in PG production, although other gram-negative bacteria that produce PGs have also been reported, including *Streptomyces coelicolor A3* (34), *Streptomyces lividans* (35), *Hahella chejuensi* KCTC 2396 (30), *Pseudovibrio denitriccans* (36) and *Pseudoalteromonas rubra* (37) (Table 2;8,9,26,27,38–50). The *S. marcescens* is a gram-negative bacterium belonging to Enterobacteriaceae. The *S. marcescens* can grow in many environments, including water, soil, plant surfaces, and insect and vertebrate digestive tracts (28). Optimal conditions for cultivating *Serratia* sp., which has a short rod-like shape, are temperature of 10°C to 40°C, pH of 5 to 9, and NaCl concentration 0% to 7% (w/v). Clinical studies indicate that approximately 20% of *S. marcescens* and 61% of *S. rubidaea* produce red pigments. Electron microscope studies indicate that some *Serratia* sp. migrate by peritrichous flagella. Some *Serratia* sp. without flagella can migrate on alga media, which enables nutritional uptake by serrawettin secretion (29).

For PG quantification, the supernatant of a culture broth (0.5 ml) was mixed with an equal volume of 2% (w/v) alum placed in a vial. Four milliliters of methanol was added to the vial and the mixture was vigorously shaken. The solution was then centrifuged at 1200 × *g* for 10 min. The supernatant was filtrated through a 0.45-μm filter to analyze PG production. 20 ml of the sample was then analyzed with HPLC on a RP-18 column. Next, chromatography was performed isocratically at a flow rate of 1 mL/min with methanol/10 mM triethylamine [19/1, v/v] (pH = 6.0) as the mobile phase. The PG was monitored at 533 nm by UV/VIS detector.

PRODIGIOSIN FERMENTATION IN MICROORGANISMS

Most of the literature on PGs focuses on medical applications, i.e., anti-tumor potential (4,32). To elucidate how PGs produce microorganisms, this section discusses several aspects of PGs production, including cultivation conditions, medium compositions and fermentation strategies.

TABLE 2. Microbial production systems of PGs and corresponding PGs types.

PG types	Strains	Refs
Prodigiosin	*Serratia marcescens*	8,9
	Serratia plymuthica	38
	Hahella chejuensis	30
	Pseudomonas magnesiorubra	39
	Vibrio psychroerythreus	26
	Streptomyces griseoviridis	27
	Vibrio gazogenes	40
Undecylprodigiosin	*Serratia marcescens* SS-1	41
	Serratia marcescens SMΔR	42
	Streptomyces coelicolor A3(2)	34
	Streptomyces lividans.	35
	Saccharopolyspora sp. nov.	43
	Streptomyces werraensis 1365T	44
Cycloprodigiosin	*Pseudoalteromonas denitrificans*	36
Nonylprodigiosin	*Actinomadura pelletieri*	45
	Actinomadura madurae	46
Butyl-meta-cycloheptylprodiginine	*Saccharopolyspora* sp. nov.	43
	Streptomyces coelicolor A3(2)	47
Butyl-cycloheptylprodiginine	*Actinomycetes*	48
Metacycloprodigiosin	*Streptomyces spectabilis* BCC 4785	49
	Streptomyces longisporus ruber	50

Cultivation conditions for PGs production Currently, the major bacteria used for PG production is *S. marcescens*. Production of PGs is inhibited at temperatures higher than 37°C and lowers than 20°C (Table 3A) (51–53). Cacace and Mazza further indicated that temperatures higher than 50°C can degrade production (54).

Studies indicate that dissolved oxygen is an essential factor in PG production in *S. marcescens* (55). The PG production in *S. marcescens* reportedly varies according to agitation speed (Table 3A). Wei and Chen indicated that PG production in *S. marcescens* was maximal at an agitation rate of 200 rpm (42). However, at higher agitation rates, PG production in *S. marcescens* was inhibited.

The pH status is also an important factor in PG production in *S. marcescens*. Therefore, maintaining a stable pH is essential for PG production in *S. marcescens* (Table 3A) (56,57). Another report indicated that *S. marcescens* produces PGs when the pH of the culture media is 3 to 7; otherwise, PGs cannot be synthesized by *S. marcescens* (58).

The PGs are also photosensitive pigments. Production of PGs reportedly varies according to the light intensity used to cultivate *S. marcescens* (59). For example, PG production is optimal when *S. marcescens* is cultured in darkness (Table 3A) (60).

Media formulations for PGs production When producing biosurfactants (e.g., serrawettin) or immunosuppressive pigments, complex media (e.g., LB broth, nutrient broth and glycerol/peptone medium) are recommended to optimize the growth and metabolic activity of *S. marcescens* species (e.g., PGs) (Table 3B) (32,42,52). Yeast extract apparently contributes to PG production in *S. marcescens.* Moreover, carbon/nitrogen ratio affects PGs production (42). Wei and Chen attempted to adjust the carbon/nitrogen ratio of yeast extract and trypton in LB broth to improve PG production. A modified LB broth reportedly improves PGs production in *S. marcescens* (41,42).

High NaCl concentrations in culture media reportedly inhibit PGs production in *S. marcescens* due to osmosis (Table 3B) (42,52,61). Salt stress is also known to affect PGs production in NaCl concentrations exceeding 1.2 M (61). At high (2.5%) salt concentrations, however, *Streptomyces coelicolor* A3(2) activates PGs production (62).

Trace elements are particularly helpful to PGs biosynthesis in *S. marcescens*, e.g., thiamine (63) and ferric acid (64), whereas light, inorganic phosphate, and ribose reportedly inhibit PGs biosynthesis (65). The inorganic phosphate (Pi) was found to reduce the PGs production by non-proliferation cells of *S. marcescens* (65). The inhibition mechanism of inorganic phosphate (Pi) was to diminish alkaline phosphatase activity, and demonstrated not to involve in the accumulation of trace elements such as iron and zinc. The adenosine triphosphate (ATP) is also a strong inhibitor of PGs biosynthesis (64,65). However, the inhibiting effects of ATP are still controversial in *Streptomyces coelicolor* A3(2). The authors propose that extracellular ATP is an effecter of *S. coelicolor* A3(2) physiology and that extracellular ATP enables high-yield antibiotics production by *S. coelicolor* A3(2) (66).

Giri et al. tested a series of media, including peanut seed broth, copra seed broth, and coconut oil broth. The authors discovered that peanut seed broth profoundly enhances PGs production (52). Vegetable oils are also promising carbon sources. Wei and Chen found that vegetable oils used as a carbon sources enhance PGs production and serrawettin secretion (Table 3B) (42).

Carbohydrates are apparently poor nutrient sources. Glucose apparently represses PGs production in *S. marcescens* via the cyclic 3′ 5′-adenosine monophosphate (cAMP) inhibitor theophylline (67). A *pig* gene study by Kalivoda et al. further indicated that cAMP is a negative regulator of PGs production in *S. marcescens* (68). Other authors have also proposed that glucose used as a carbon source for *S. marcescens* affects PG production by accelerating extracellular acidification (54,55).

TABLE 3. Fermentation conditions for PGs production by microorganisms, including cultivation conditions (A), cultivation media (B) and fermentation strategies (C).

A

Strains	Culture conditions	PG production (mg/L)	Refs
	Temperature (°C)		
Serratia marcescens NIMA	25	159	51
Serratia marcescens	28	39	52
Serratia marcescens SS-1	30	30	41
Serratia marcescens	37 or >37	0	51
	Agitation speed (rpm)		
Serratia marcescens SS-1	<200	23	41
Serratia marcescens SS-1	200	30	41
Serratia marcescens SS-1	>200	17	41
Serratia marcescens	375[a]	240	55
	pH value		
Serratia marcescens	>3	0	58
Serratia marcescens SS-1	7	30	41
Serratia marcescens	8.0 or 8.5	–[a]	57
	Light intensity		
Serratia marcescens	<2000 lux	–[a]	60

B

Strains	Media types	PG production (mg/L)	Refs
Serratia marcescens	Nutrient broth	0.52	52
Serratia marcescens	Peptone glycerol broth	0.30	52
Serratia marcescens	Sesame seed broth	16.68	52
Serratia marcescens	Nutrient broth with 0.5% maltose	1.84	52
Serratia marcescens	Nutrient broth with 0.5% glucose	1.69	52
Serratia marcescens	Sesame seed broth with 0.5% maltose	9.43	52
Serratia marcescens	Sesame seed broth with 0.5% glucose	1.47	52
Serratia marcescens	Peanut seed broth	38.75	52
Serratia marcescens	Copra seed broth	1.94	52
Serratia marcescens	Peanut oil broth	2.89	52
Serratia marcescens	Coconut oil broth	1.42	52
Serratia marcescens	Sesame oil broth	1.01	52
Serratia marcescens SMΔR	Modified LB broth with 6% soybean oil	198.00	42
Serratia marcescens SMΔR	Modified LB broth with 6% sunflower oil	790.00	42
Serratia marcescens SMΔR	Modified LB broth with 6% olive oil	453.00	42
Serratia marcescens SS-1	Modified LB broth with 10 g/L Pro	2500.00	41
Serratia marcescens SS-1	Modified LB broth with 5 g/L His	1400.00	41
Serratia marcescens SS-1	Modified LB broth with 2.5 g/L Asp	1500.00	41

C

Strains	Fermentation strategies	PG production (mg/L)	Refs
Serratia marcescens SMΔR	Batch culture by C/N ratio	152.00	42
Serratia marcescens SS-1	Batch culture by C/N ratio	1500.00	41
Serratia marcescens SMΔR	Batch culture by vegetable oils	790.00	42
Serratia marcescens	Batch culture by vegetable oils	1.01	41
Serratia marcescens Nima	Batch culture by precursors	22.10	42
Serratia marcescens SS-1	Batch culture by precursors	2500.00	41
Serratia marcescens	Batch culture by seed broth	3900.00	52
Serratia marcescens UCP1459	Batch culture by renewable-resources	49500.00	68
Serratia marcescens spp.	Batch culture with immobilization	0.93[a]	77
Serratia marcescens NIMA	Batch culture with immobilization	1000.00	87
Streptomyces coelicolor A3(2)	Continuous culture	0.81	88
Serratia sp. KH-95	Bioreactor with *in situ* separation	42000.00	84
Serratia sp. KH-95	Bioreactor with *in situ* separation	13100.00	85
Serratia sp. KH-95	Bioreactor with *in situ* separation	69200.00	83
Hahella chejuensis KCTC 2396	Statistical optimization	1198.00	78
Hahella chejuensis M3349	Genetic engineering and statistical optimization	2600.00	79
Serratia marcescens O8	PG recovery by SDS	7.00[a]	89

[a] The unit of PGs production not used in mg/L.

However, PGs production in a nutrition broth can be enhanced significantly by adding 0.5% glucose or 0.5% maltose (Table 3B) (52).

Williams et al. indicated uptake of five-carbon amino acids such as aspartate, proline, glutamate and ornithine by *S. marcescens* promotes PGs production. However, although alanine is conducive to PG biosynthesis, whether it affects PGs biosynthesis is unknown. Reports also indicate that methionine and methionine analogues do not affect PG biosynthesis (69). William further indicated that proline uptake does not occur in *S. marcescens*, whereas *S. marcescens* directly incorporate proline into the 4-methoxy-2,2′-bipyrrole-5-carbaldehyde (MBC) structure of the PPM skeleton to increase PGs production (47,70,71). The mechanism of proline incorporation in PGs synthesis was recently elucidated (Table 3C) (41,71). Monopyrrole is the cause of differences in biosynthesis between PG and its analogues. Various monopyrrole types condense to PGs structures in *S. marcescens* (32).

Some wastes such as industrial wastewater and food waste can be used as nutrients for production of bio-products by microorganisms. De Araújo et al. added 6% cassava wastewater into medium. Their results showed that adding 6% cassava wastewater enhanced PGs production and reduced production costs (72).

Fermentation strategies for PGs production The various fermentation strategies used for bio-product production by microorganisms include genetic engineering, batch culture, continuous culture, bioreactor culture, and immobilization culture. Bio-product production can also be enhanced by applying statistical methods such as response surface methodology. Most fermentation strategies for PGs production apply batch culture. Therefore, this section describes the above fermentation strategies but not batch culture strategies, which are discussed in the section on culture conditions and medium formulations.

Gene expressions of microorganisms vary rapidly with environmental hints such as cell proliferation, cultivation temperature and cultivation medium. The gene expression of PG is a quorum sensing mechanism. Quorum sensing system is a cell to cell interaction system that either increases or decreases PG production by high or low cell density (42). The PGs are peptide-antibiotics synthesized by non-ribosomal peptide synthases (NRPSs). The mechanism of NRPS synthesis is the intermediate covalent compound of phosphopantetheine, which synthesizes peptide chains from peptide synthesis enzymes (73). The *pig* gene cluster that controls PGs biosynthesis has been identified in *S. marcescens* ATCC 274 and in *Serratia* sp. ATCC 39006 (Fig. 2) (73–75). Fourteen open reading frames in *pig* gene cluster in *S. marcescens* ATCC 274, i.e., *pig* A to *pig* N, can be translated into a single polycistronic mRNA. *Pig* C is responsible for synthesizing condensed enzymes. *Pig* B, D and E regulate monopyrrole (2-methyl-3-n-amyl-pyrrole, MAP) synthesis. *Pig* F to N participates in MBC synthesis directly and indirectly, respectively (73,76). Therefore, the tripyrrole pigment compounds, PGs, are synthesized by condensing enzyme when the pigment precursors (i.e., MAP and MBC) appear (Fig. 3) (73).

Dauenhauer et al. indicated that cloning the *Sau 3A* DNA fragment of *S. marcescens* Nima to *E. coli* K-12 by the cosmid vector pHC79 promotes secretion of PGs by *E. Coli* K-12 (76). Moreover, the mutant resulting from transposon mutation of *S. marcescens* ATCC 274 profoundly enhances PGs production (77). Kim et al. performed a statistical method of optimizing PGs production (2.6 g/L) in an a mutant strain, i.e., Hahella chejuensis M3349 (78). The related genes-*pswP and hexS* reportedly exhibit parallel production of serrawettin and PGs (79,80). Recently, Borodina et al. found that phosphofructokinase deletion in *Streptomyces coelicolor* A3(2) can increase PGs production (81).

Continuous cultivation is rarely performed in the laboratory. Crucial advantages such as shortening the fermentation period, improving the consistency of bio-products, reducing losses of bio-products and protecting the environment become obvious when continuous cultivation is performed. The continuous culture technique is generally limited to the exponential phase of batch culture of a microorganism to provide an environment with minimal variation in nutrients and biomass. Kang et al. evaluated the effects of growth rate and nutrient feed rate on PGs production in *Streptomyces coelicolor* A3(2) by continuous culture (82) (Table 3C). Their experimental results indicated that PG production in the relA mutant was lower than that of the parental strain under conditions of glucose and ammonium limitation. The specific rate of PGs production was enhanced by adding glucose but suppressed by adding phosphate (82).

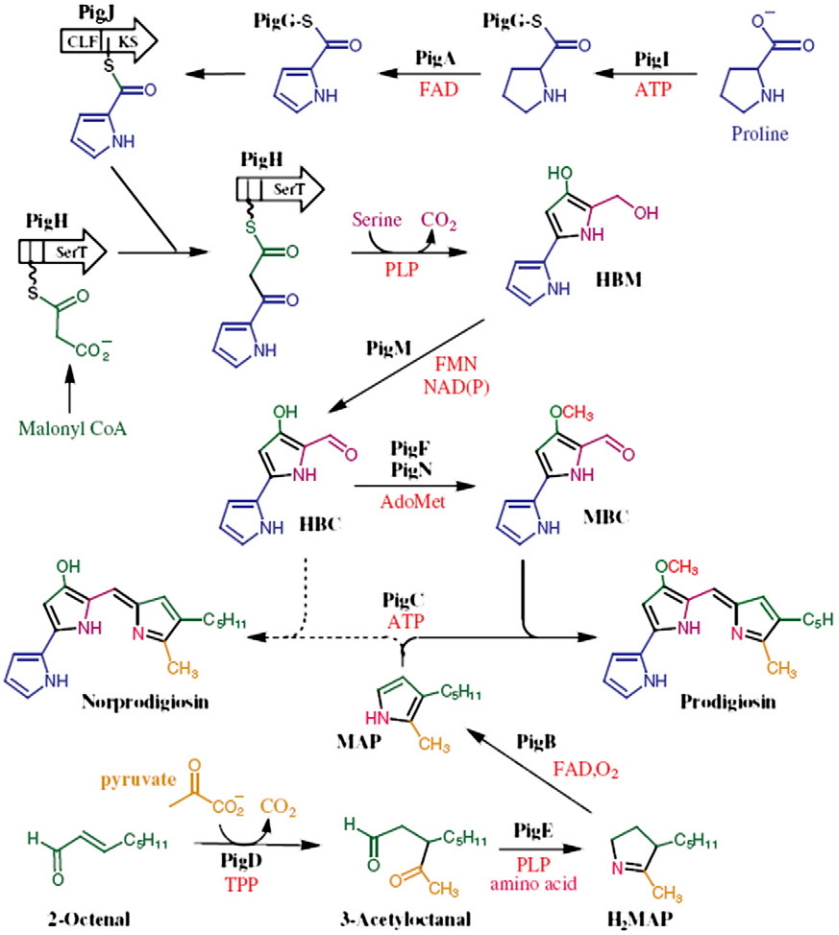

FIG. 2. Biosynthetic pathway of PGs (75).

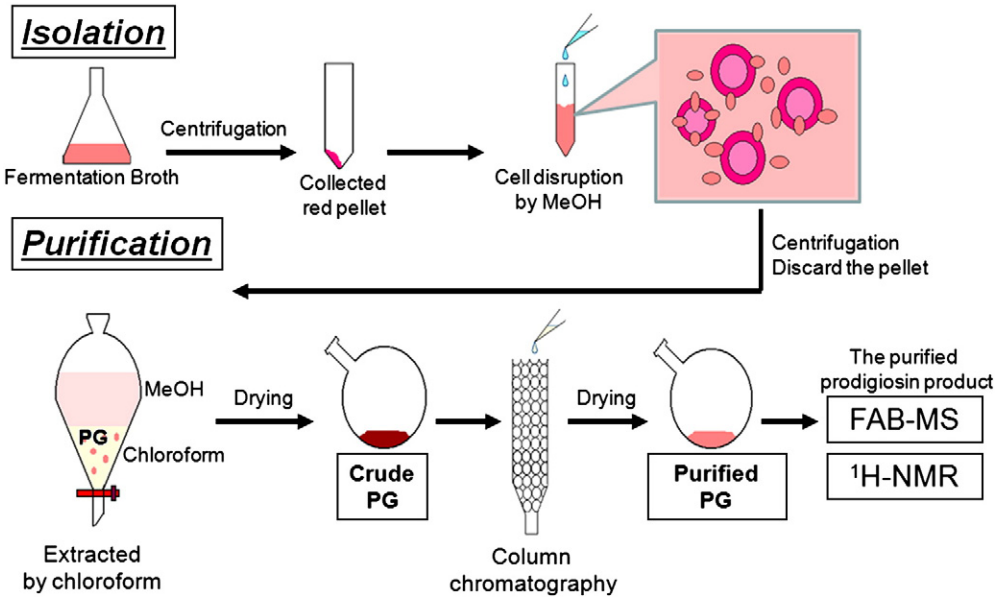

FIG. 3. Processing schemes for isolating and purifying PGs.

The PGs production is limited in batch cultivation due to product feedback inhibition (83–86). This phenomenon also occurs in the culture of other secondary metabolites. Kim et al. developed an *in situ* bioreactor with an external extraction column for recovering PGs. The PG product could be removed after performing membrane filtration, extraction, evaporation, and adsorption processes to prevent end-product inhibition. Their experimental results showed that the increase in PG production was 1.31-fold higher than that in the simple batch culture (84,85). To improve the recovery yield of PG, Bae et al. further fabricated an internal extraction column for recovering PGs. The PG production was 1.8-fold higher than that obtained in an external extraction column (Table 3C) (86).

Immobilization culture in biotechnology applications is performed to immobilize the bacteria in a special carrier in order to enhance bacterial proliferation and bio-product production. Two studies have applied this concept to enhance PGs production. Yamashita et al. added a silica gel carrier to the cultivation medium. They reported that red bacteria observed on silica particles significantly enhanced production of biomass, PGs and serrawettin (77). Syzdek et al. also used aerosols to immobilize bacteria in bubbles. Their experimental results showed increased biomass and PGs (87).

The common experimental procedure is to optimize a single factor and to maintain the remaining factors. Variation in other factors should not be considered in the same run. Therefore, the experimental results in this study do not depict the combined effects of all the factors involved. The optimal values from these experiments are difficult to determine due to the large number of experiments. All the factors can be optimized by a statistical experimental design such as response surface methodology (RSM). Kim et al. was the first research group to enhance PGs production in *S. marcescens* KCTC 2396 by using statistical methods to optimize the medium composition. They reported higher PGs production in the experimental group than in the control group (Table 3C) (78,79).

PURIFICATION AND ISOLATION OF PRODIGIOSINS

PGs purification and characterization Crude PGs traditionally extracted from *S. marcescens* or *Streptomyces* spp. by various solvents such as methanol, acetone, and chloroform (26,41,42,51). The PGs have four to six fractions in the crude product obtained by solvent extraction (88). Conventional methods of separating various fractions of PGs use column chromatography such as silica gel (26). Fractions of varying color, such as orange-red fraction, pink fraction and purple fraction, can be eluted out by passing the silica gel (26,41,42,88). The orange-red fraction is collected and dried in a vacuum oven. Finally, a viscoid red powder with high purity is harvested (Fig. 3). However, conventional PG purification methods use too many organic solvents, possibly resulting in the cytotoxicity of PGs.

The molecular weight and chemical structure of the purified product was characterized by mass spectrometry and NMR. The purified product was dissolved in D-chloroform ($CDCl_3$) and the solution then was analyzed by NMR to identify the structure of the purified product. The purified product dissolved in methanol was injected into the FAB-MS to determine the molecular weight of red pigment.

Surfactant for enhanced PGs recovery The literature shows that adding sodium dodecyl sulfate into medium enhances PGs production due to an increase in negative binding sites by sodium dodecyl sulfate with cell envelope components (Table 3C) (89). Mallick further indicated that *S. marcescens* with high cell surface hydrophilicity produce PGs (90). Therefore, we hypothesize that serrawettin production by *S. marcescens* affects the release of PG from *S. marcescens.* Yamashita et al. noted that the metabolic control system involved in serrawettin production resembles that in PG production (77). Furthermore, the *pswP* gene reportedly affects the parallel production of PGs and serrawettin W1 in *S. marcescens* (80). Wei and Chen proposed that PGs production correlates with extracellular surface emulsification activity, i.e., PG production is linked to serrawettin (42).

Direct extraction after bioreactor fermentation Several literatures showed bacteria cultivated by *in situ* bioreactor with extraction absorbent column to recover PGs in order to prevent end-product inhabitation and to increase PGs production (83,84). Kim et al. first reported the development of a bioreactor with external column separation (84). Three adsorbents, HP-20, SP-850, and XAD-16, were evaluated in terms of capacity to bind pigment efficiently. Resin SP-850 had a higher capacity compared to HP-20 and XAD-16 (84). Bae et al. further developed a bioreactor with internal absorbent. Efficiency of PGs recovery and production was highest in the bioreactor with external absorbent (86). Wang et al. (14) improved PGs release from the cell envelope by adding Tween 80 and used an adsorbent (X-5 resin) for direct recovery of PGs from culture broth by

static adsorption or *in situ* separation. The recovery yield (83%) in that study was much higher than that obtained by conventional method (50%) (88).

ANTICANCER ACTIVITY OF PGs

PGs, including PG, undecylprodigiosin and cycloprodigiosin, are cytotoxic against a wide range of human cancer cell lines while sparing nonmalignant cells (33,91). Because of this selective cytotoxicity, PGs are promising anticancer agents. The *in vivo* anticancer activity of PGs was first demonstrated by the inhibitory effect of cycloprodigiosin on xenografted Huh-7 hepatocarcinoma cells (92). A mouse melanoma model also showed that prodigiosin elicits an anti-metastatic effect by reducing lung metastasis (93), which suggests that PGs stall cancer progression through multiple mechanisms.

Apoptosis The cytotoxicity of PGs is mainly attributable to their proapoptotic effect against malignant cells, which is independent of p53 and drug resistance (94–96). Accumulating evidence has started to unravel the multiple pathways whereby PGs induce apoptosis (Fig. 4). The modes of action and molecular targets responsible for the proapoptotic effect of PGs are discussed below.

Acidification of intracellular pH (pHi) is apparently a key trigger of apoptosis (97). In this regard, prodiginines lower pHi by facilitating H^+/Cl symport across vesicular membranes to the cytosol (98). Intracellular acidification is essential for cycloprodigiosin to provoke apoptosis in HL-60 cells (99) but is dispensable in PG-induced colon cancer cell apoptosis (101). These findings suggest that the role of intracellular acidification in PGs-induced apoptosis is in PGs- or cell type-dependent manners.

Members of the BCL-2 family are pivotal to the initiation of mitochondrial apoptotic pathway by regulating the permeability of the outer mitochondrial membrane (OMM). Increase in OMM permeability leads to the cytosolic release of cytochrome c for the activation of caspase 9-initiated caspase cascade to induce apoptosis. The balance between antiapoptotic and proapoptotic BCL-2 proteins therefore acts as a life-or-death switch (102). Prodigiosin is known to up-regulate the proapoptotic BCL-2 protein BAX in MCF-7 cells (95). Similarly, we demonstrated earlier that undecylprodigiosin reduces antiapoptotic BCL-$_X$L but elevates proapoptotic BIK, BIM, MCL-1S and NOXA (96). Altogether, modulating the BCL-2-based life-or-death switch is fundamental for PGs to induce apoptosis.

Survivin and XIAP are endogenous inhibitors of caspases. Overexpression of survivin or XIAP, which confers drug resistance, is common in cancer cells (103,104). An earlier study by the authors found that both survivin and XIAP are reduced by undecylprodigiosin in MCF-7 cells (96). We further revealed that PG transcriptionally down-regulate survivin to achieve synergistic toxicity with paclitaxel, a common chemotherapeutic known to up-regulate survivin (105). Intriguingly, down-regulation of survivin and XIAP may partly explain the selective toxicity of PGs against cancer cells, as cancer cells are highly dependent on survivin and/or XIAP to withstand the high basal levels of apoptotic stress (106,107).

The tumor suppressor protein p53 is a master trigger of apoptosis. Most cancer cells carry functionally defective p53 and are therefore more resistant to chemotherapy-induced apoptosis (108). Notably, PGs trigger

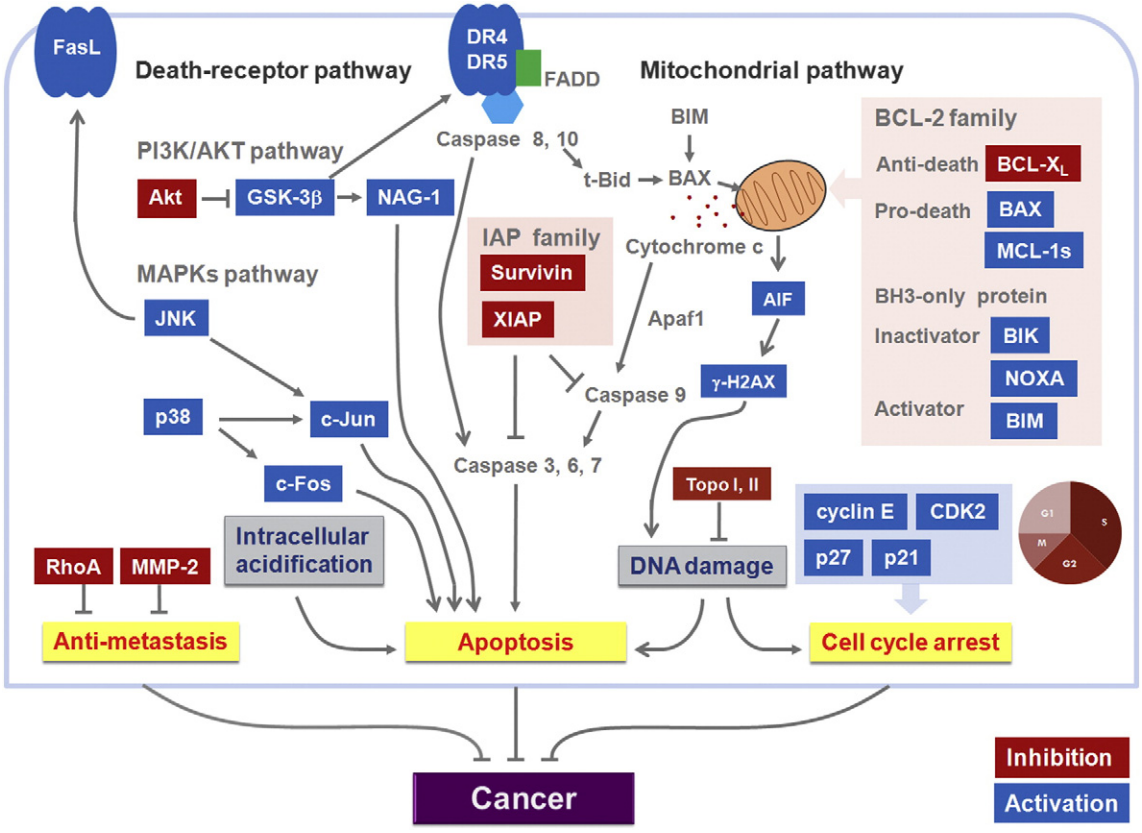

FIG. 4. Anti-tumor mechanisms of PGs. PGs exert their anti-tumor effect by inducing apoptosis, cell cycle arrest, or inhibition of metastasis. PGs modulate the expression of BCL-2 family proteins, IAP proteins (survivin and XIAP), and death ligand/receptors to engage both mitochondrial and death-receptor apoptotic pathways. Suppression of CDK activities and down-regulation of RhoA and MMP-2 account for the cell cycle arrest and anti-metastatic effects of PGs, respectively. Signaling pathways mediated by JNK, p38-MAPK and PI3K/AKT/GSK-3β are all involved in PGs-induced apoptosis. See text for details.

apoptosis in cell lines irrespective of p53 status (94–96). The dispensable role of p53 in PGs-induced apoptosis was further validated by our finding that both MCF-7 cells and their p53-knockdown counterparts showed comparable sensitivity to undecylprodigiosin (96). Induction of p53-independent apoptosis is actually an advantage of PGs over other chemotherapeutics whose proapoptotic effects require functional p53.

Cells manage DNA lesions by inducing cell cycle arrest for DNA repair or apoptosis if DNA is severely damaged. PG facilitates double-stranded DNA (dsDNA) cleavage in the presence of copper, and this copper-mediated nuclease activity is essential for PG-induced apoptosis (109). Additionally, PG perturbs DNA structure by direct DNA intercalation and also by inhibiting the activity of both topoisomerase I and II to cause dsDNA breaks (110).

Signaling cascades mediated by the family of mitogen-activated protein kinases (MAPKs), including ERK, JNK and p38-MAPK, contribute to PGs-induced apoptosis. In HL-60 cells, cycloprodigiosin induced JNK activation, leading to c-Jun phosphorylation and FasL up-regulation (99). In contrast, PG induced the phosphorylation of p38-MAPK instead of JNK in Jurkat cells (100), and this apoptosis was suppressed by the protein kinase C (PKC)-MEK-ERK pathway activated by phorbolmyristate acetate (PMA), even though PG did not induce ERK phosphorylation (111). Recently, we unraveled that undecylprodigiosin induces phosphorylation/activation of both JNK and p38-MAPK in T-47D cells, but it is the JNK-mediated signaling that predominantly mediates undecylprodigiosin-induced apoptosis (manuscript in preparation).

The phosphatidylinositol 3-kinase (PI3K)-AKT-glycogen synthase kinase-3β (GSK-3β) pathway is also central to the proapoptotic activity of PG. Specifically, PG-induced AKT dephosphorylation activates GSK-3β which up-regulates death receptors 4 and 5 as well as nonsteroidal anti-inflammatory drug-activated gene 1 (NAG-1), a proapoptotic protein (112). Importantly, GSK-3β is required for PG-induced apoptosis, whose level is suppressed by inhibition of GSK-3β activity.

Cell cycle arrest Aside from inducing apoptosis of cancer cells, PGs also halt cancer growth by provoking cell cycle arrest at non-cytotoxic concentrations. To this end, undecylprodigiosin reduces T lymphocyte proliferation by inhibiting CDK4 and CDK2 (113). Similarly, PG induces growth arrest of Jurkat cells at the G1-S phase transition by reducing Rb phosphorylation through the inhibition of the kinase activity of cyclin E-CDK2 and cyclin A-CDK2, as well as by up-regulating $p21^{CIP1/WAF1}$ and $p27^{KIP1}$ (114).

A recent study by Soto-Cerrato et al. (115) provided a mechanistic insight into how PG up-regulates $p21^{CIP1/WAF1}$. In MCF-7 cells, non-cytotoxic doses of PG transcriptionally up-regulate $p21^{CIP1/WAF1}$, but the up-regulation is independent of p53. Instead, PG engages the TGF-β signaling pathway to promote $p21^{CIP1/WAF1}$ expression, as blockade of TGF-β receptor abolishes $p21^{CIP1/WAF1}$ induction by PG (115).

Anti-invasion and anti-metastasis Distant metastases are a leading cause of cancer mortality, and contribute to the demand for anti-metastasis drugs. The therapeutic benefits of PG have been demonstrated in a mouse melanoma model by reduced lung metastasis and a corresponding increase in survival (93). Mechanistically, this anti-metastatic activity of PG is likely attributable to its inhibiting effect on cell migration and cell invasion, possibly through down-regulation of RhoA and matrix metalloproteinase-2 (MMP-2) (93).

The potent anticancer activities of some PG family members are now evident. The selective cytotoxicity against cancer cells, p53-independent proapoptotic effect, and anti-metastatic activity of PGs indicate the great potential of these bacterial metabolites as anticancer agents. Recent multiple phase I and II oncology trials of GX15-070 (obatoclax), a synthetic analogue of PG developed by Gemin X Pharmaceuticals, further substantiates the use of PGs as lead compounds to develop novel cancer therapeutics (33,91). Further studies are needed to determine how PGs induce apoptosis, cell cycle arrest, and anti-metastasis, as improved understanding of the activities of these drugs can enable the development of novel and effective cancer therapeutics.

FUTURE PROSPECTS

Most of the commercially available anti-tumor drugs are chemically synthesized and have substantial side effects. Therefore, pharmaceutical factories and researchers focus on naturally occurring anti-tumor drugs to replace chemical synthetic drugs. One of the naturally occurring anti-tumor drugs is pigments extracted from vegetables and fruits, such as β-carotene, lycopene, curcumin and anthocyanins. Although these pigments have potent anti-tumor and anti-oxidant properties with minimal side effects, extraction costs of these pigments are very high. Alternatively, another naturally occurring anti-tumor drug is red pigments extracted from various microorganisms, e.g., PG. The merits of PGs in anti-tumor drug development include only a shorter preparation time and a different pathway for anti-tumor treatment than other natural drugs, but also the immunosuppressive, antifungal, antiproliferative, and proapoptotic properties of PGs. Conversely, facts including high production costs and the complex purification and isolation processes from the fermentation broth of various microorganisms limit the development of PGs as anti-tumor drugs. Hence, several issues must be addressed to improve PGs production in the future. First, PGs production should be further explored on other potential stains in addition to *S. marcescens*. Second, the defined medium must be developed for industrial applications. Third, a feasible fermentation strategy, e.g., two-stage fermentation and fed-batch fermentation, is essential for application to PG production. Fourth, the bioreactor plus recovery system should be further developed in terms of feedback inhibition of PGs. Finally, purification of PGs was also further improved with the HPLC separation system to reduce the solvent used and to reduce the cytotoxicity by purification.

Researchers are now recognizing the potent anticancer activity of some PGs family members. The combination of selective cytotoxicity against cancer cells, p53-independent proapoptotic effect, and anti-metastatic activity of PGs endows these bacterial metabolites with great potential as anticancer agents. Future studies should explore the mechanisms whereby PGs induce cell cycle arrest, apoptosis and anti-metastasis, as detailed understanding of the activities of these drugs hold great promise for rational design of novel and effective cancer therapeutics. According to the reasons mentioned above, to develop fermentation process of PGs production by microorganisms is necessary.

ACKNOWLEDGMENTS

This review article is contributed by Dr. Yu-Hong Wei as the recipient of the Young Asian Biotechnologist Prize in 2010. The authors would like to thank the National Science Council of the Republic of China, Taiwan, for partially supporting this research under Contract Numbers NSC 98-2221-E-155-026 and NSC 99-2627-M-155-001.

References

1. **Takahashi, H., Aoyagi, K., Nakanishi, Y., Sasaki, H., Yoshida, T., and Honda, H.:** Classification of intramural metastases and lymph node metastases of esophageal cancer from gene expression based on boosting and projective adaptive resonance theory, J. Biosci. Bioeng., **102**, 46–52 (2006).
2. **Tokumitsu, A., Wakitani, S., and Takagi, M.:** Noninvasive discrimination of human normal cells and malignant tumor cells by phase-shifting laser microscopy, J. Biosci. Bioeng., **109**, 499–503 (2010).
3. **Souweidane, M. M., Krieger, M. D., Weiner, H. L., and Finlay, J. L.:** Surgical management of primary central nervous system germ cell tumors: proceedings from the Second International Symposium on Central Nervous System Germ Cell Tumors, J. Neurosurg. Pediatr., **6**, 125–130 (2010).
4. **Kim, K. Y., Chung, H. C., and Rha, S. Y.:** A weighted sample size for microarray datasets that considers the variability of variance and multiplicity, J. Biosci. Bioeng., **108**, 252–258 (2009).

5. **Ma, X. and Wang, Z.:** Anticancer drug discovery in the future: an evolutionary perspective, Drug Discov. Today, **14**, 1136–1142 (2009).
6. **DeVita, V. T., Jr. and Chu, E.:** A history of cancer chemotherapy, Cancer Res., **68**, 8643–8653 (2008).
7. **Chabner, B. A. and Roberts, T. G., Jr.:** Timeline: chemotherapy and the war on cancer, Nat. Rev. Cancer, **5**, 65–72 (2005).
8. **Cragg, G. M., Grothaus, P. G., and Newman, D. J.:** Impact of natural products on developing new anti-cancer agents, Chem. Rev., **109**, 3012–3043 (2009).
9. **Ravelo, A. G., Estévez-Braun, A., Chávez-Orellana, H., Pérez-Sacau, E., and Mesa-Siverio, D.:** Recent studies on natural products as anticancer agents, Curr. Top. Med. Chem., **4**, 241–265 (2004).
10. **Liu, J., Gunn, L., Hansen, R., and Yan, J.:** Yeast-derived beta-glucan in combination with anti-tumor monoclonal antibody therapy in cancer, Recent Pat. Anticancer Drug Discov., **4**, 101–109 (2009).
11. **Robey, R. W., Obrzut, T., Shukla, S., Polgar, O., Macalou, S., Bahr, J. C., Di Pietro, A., Ambudkar, S. V., and Bates, S. E.:** Becatecarin (rebeccamycin analog, NSC 655649) is a transport substrate and induces expression of the ATP-binding cassette transporter, ABCG2, in lung carcinoma cells, Cancer Chemother. Pharmacol., **64**, 575–583 (2009).
12. **Bertomeu, T., Zvereff, V., Ibrahim, A., Zehntner, S. P., Aliaga, A., Rosa-Neto, P., Bedell, B. J., Falardeau, P., and Gourdeau, H.:** TLN-4601 peripheral benzodiazepine receptor (PBR/TSPO) binding properties do not mediate apoptosis but confer tumor-specific accumulation, Biochem. Pharmacol., **80**, 1572–1579 (2010).
13. **Kale, A. J., McGlinchey, R. P., and Moore, B. S.:** Characterization of 5-chloro-5-deoxy-D-ribose 1-dehydrogenase in chloroethylmalonyl coenzyme A biosynthesis: substrate and reaction profiling, J. Biol. Chem., **285**, 33710–33717 (2010).
14. **Wang, C., Wesener, S. R., Zhang, H., and Cheng, Y. Q.:** An FAD-dependent pyridine nucleotide-disulfide oxidoreductase is involved in disulfide bond formation in FK228 anticancer depsipeptide, Chem. Biol., **16**, 585–593 (2009).
15. **Kumaran, R. S., Kim, H. J., and Hur, B. K.:** Taxol promising fungal endophyte, Pestalotiopsis species isolated from *Taxus cuspidata*, J. Biosci. Bioeng., **110**, 541–546 (2010).
16. **Sakai, T., Shindo, K., Odagawa, A., Suzuki, A., Kawai, H., Kobayashi, K., Hayakawa, Y., Seto, H., and Otake, N.:** Absolute configuration of spicamycin, an antitumor antibiotic produced by *Streptomyces alanosinicus*, J. Antibiot. (Tokyo), **48**, 899–900 (1995).
17. **Yamamoto, M., Takakura, A., and Masuda, N.:** Next-generation anthracycline for the management of small cell lung cancer: focus on amrubicin, Drug Des. Dev. Ther., **2**, 189–192 (2009).
18. **Pérez, M., Crespo, C., Schleissner, C., Rodríguez, P., Zúñiga, P., and Reyes, F.:** Tartrolon D, a cytotoxic macrodiolide from the marine-derived actinomycete *Streptomyces* sp. MDG-04-17-069, J. Nat. Prod., **72**, 2192–2194 (2009).
19. **Konishi, M., Sugawara, K., Kofu, F., Nishiyama, Y., Tomita, K., Miyaki, T., and Kawaguchi, H.:** Elsamicins, new antitumor antibiotics related to chartreusin. I. Production, isolation, characterization and antitumor activity, J. Antibiot. (Tokyo), **39**, 784–791 (1986).
20. **Pearson, L., Mihali, T., Moffitt, M., Kellmann, R., and Neilan, B.:** On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin, Mar. Drugs, **8**, 1650–1680 (2010).
21. **Jensen, P. R., Williams, P. G., Oh, D. C., Zeigler, L., and Fenical, W.:** Species-specific secondary metabolite production in marine actinomycetes of the genus *Salinispora*, Appl. Environ. Microbiol., **73**, 1146–1152 (2007).
22. **Thornburg, C. C., Zabriskie, T. M., and McPhail, K. L.:** Deep-sea hydrothermal vents: potential hot spots for natural products discovery? J. Nat. Prod., **73**, 489–499 (2010).
23. **Fujiwara, S.:** Extremophiles: developments of their special functions and potential resources, J. Biosci. Bioeng., **94**, 518–525 (2002).
24. **Kroiss, J., Kaltenpoth, M., Schneider, B., Schwinger, M. G., Hertweck, C., Maddula, R. K., Strohm, E., and Svatos, A.:** Symbiotic *Streptomycetes* provide antibiotic combination prophylaxis for wasp offspring, Nat. Chem. Biol., **6**, 261–263 (2010).
25. **Kumaran, R. S., Muthumary, J., and Hur, B. K.:** Taxol from *Phyllosticta citricarpa*, a leaf spot fungus of the angiosperm Citrus medica, J. Biosci. Bioeng., **106**, 103–106 (2008).
26. **D'Aoust, J. Y. and Gerber, N. N.:** Isolation and purification of prodigiosin from *Vibrio psychroerythrus*, J. Bacteriol., **118**, 756–757 (1974).
27. **Kawasaki, T., Sakurai, F., and Hayakawa, Y.:** A prodigiosin from the roseophilin producer *Streptomyces griseoviridis*, J. Nat. Prod., **71**, 1265–1267 (2008).
28. **Fukuda, K., Tamura, T., Ito, H., Yamamoto, S., Ochi, K., and Inagaki, K.:** Production improvement of antifungal, antitrypanosomal nucleoside sinefungin by rpoB mutation and optimization of resting cell system of *Streptomyces incarnatus* NRRL 8089, J. Biosci. Bioeng., **109**, 459–465 (2010).
29. **Boonlarppradab, C., Kauffman, C. A., Jensen, P. R., and Fenical, W.:** Marineosins A and B, cytotoxic spiroaminals from a marine-derived actinomycete, Org. Lett., **10**, 5505–5508 (2008).
30. **Kim, D., Lee, J. S., Park, Y. K., Kim, J. F., Jeong, H., Oh, T. K., Kim, B. S., and Lee, C. H.:** Biosynthesis of antibiotic PGs in the marine bacterium *Hahella chejuensis* KCTC 2396, J. Appl. Microbiol., **102**, 937–944 (2007).
31. **Reapoport, H. and Holden, K. G.:** The synthesis of prodigiosin, J. Am. Chem. Soc., **83**, 635–642 (1961).
32. **Fürstner, A.:** Chemistry and biology of roseophilin and the prodigiosin alkaloids: a survey of the last 2500 years, Angew. Chem. Int. Ed. Engl., **42**, 3582–3603 (2003).
33. **Pandey, R., Chander, R., and Sainis, K. B.:** Prodigiosins as anti cancer agents: living upto their name, Curr. Pharm. Des., **15**, 732–741 (2009).
34. **Sevcikova, B. and Kormanec, J.:** Differential production of two antibiotics of *Streptomyces coelicolor* A3(2), actinorhodin and undecylprodigiosin, upon salt stress conditions, Arch. Microbiol., **181**, 384–389 (2004).
35. **Avignone Rossa, C., White, J., Kuiper, A., Postma, P. W., Bibb, M., and Teixeira de Mattos, M. J.:** Carbon flux distribution in antibiotic-producing chemostat cultures of *Streptomyces lividans*, Metab. Eng., **4**, 138–150 (2002).
36. **Sertan-de Guzman, A. A., Predicala, R. Z., Bernardo, E. B., Neilan, B. A., Elardo, S. P., Mangalindan, G. C., Tasdemir, D., Ireland, C. M., Barraquio, W. L., and Concepcion, G. P.:** *Pseudovibrio denitrificans* strain Z143-1, a heptylprodigiosin-producing bacterium isolated from a Philippine tunicate, FEMS Microbiol. Lett., **277**, 188–196 (2007).
37. **Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqbal, M. M., Yumoto, I., Ezura, Y., and Christen, R.:** *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent of red spot disease of Laminaria japonica, Int. J. Syst. Bacteriol., **48**, 769–774 (1998).
38. **Berg, G.:** Diversity of antifungal and plant-associated *Serratia plymuthica* strains, J. Appl. Microbiol., **88**, 952–960 (2000).
39. **Gerber, N. N.:** Prodigiosin-like pigments. CRC, Crit. Rev. Microbiol., **3**, 469–485 (1975).
40. **Allen, G. R., Reichelt, J. L., and Gray, P. P.:** Influence of environmental factors and medium composition on *Vibrio gazogenes* growth and prodigiosin production, Appl. Environ. Microbiol., **5**, 1727–1732 (1983).
41. **Wei, Y. H., Yu, W. J., and Chen, W. C.:** Enhanced undecylprodigiosin production from *Serratia marcescens* SS-1 by medium formulation and amino-acid supplementation, J. Biosci. Bioeng., **100**, 466–471 (2005).
42. **Wei, Y. H. and Chen, W. C.:** Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SMΔR by medium improvement and oil-supplementation strategies, J. Biosci. Bioeng., **99**, 616–622 (2005).
43. **Liu, R., Cui, C. B., Duan, L., Gu, Q. Q., and Zhu, W. M.:** Potent in vitro anticancer activity of metacycloprodigiosin and undecylprodigiosin from a sponge-derived actinomycete *Saccharopolyspora* sp. nov., Arch. Pharm. Res., **28**, 1341–1344 (2005).
44. **Rusanova, E. P., Alekhova, T. A., Fedorova, G. B., and Katrukha, G. S.:** An antibiotic complex produced by *Streptomyces werraensis* 1365T strain, Prikl. Biokhim. Mikrobiol., **36**, 564–568 (2000).
45. **Gerber, N. N.:** Prodigiosin-like pigments from *Actinomadura (Nocardia) pelletieri*, J. Antibiot. (Tokyo), **24**, 636–640 (1971).
46. **Gerber, N. N.:** Prodigiosin-like pigments from *Actinomadura* (Nocardia) *pelletieri* and *Actinomadura madurae*, Appl. Microbiol., **18**, 1–3 (1969).
47. **Cerdeño, A. M., Bibb, M. J., and Challis, G. L.:** Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3(2): new mechanisms for chain initiation and termination in modular multienzymes, Chem. Biol., **8**, 817–829 (2001).
48. **Gerber, N. N. and Lechevalier, M. P.:** Prodiginine (prodigiosin-like) pigments from *Streptomyces* and other aerobic *Actinomycetes*, Can. J. Microbiol., **22**, 658–667 (1976).
49. **Isaka, M., Jaturapat, A., Kramyu, J., Tanticharoen, M., and Thebtaranonth, Y.:** Potent in vitro antimalarial activity of metacycloprodigiosin isolated from *Streptomyces spectabilis* BCC 4785, Antimicrob. Agents Chemother., **46**, 1112–1113 (2002).
50. **Wasserman, H. H., Rodgers, G. C., and Keith, D. D.:** Metacycloprodigiosin, a tripyrrole pigment from *Streptomyces longisporus* ruber, J. Am. Chem. Soc., **91**, 1263–1264 (1969).
51. **Qadri, S. M. and Williams, R. P.:** Induction of prodigiosin biosynthesis after shift-down in temperature of nonproliferating cells of *Serratia marcescens*, Appl. Microbiol., **23**, 704–709 (1972).
52. **Giri, A. V., Anandkumar, N., Muthukumaran, G., and Pennathur, G.:** A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil, BMC Microbiol., **4**, 1–10 (2004).
53. **Willams, R. P., Gott, C. L., and Qadri, S. M.:** Induction of pigmentation in nonproliferating cells of *Serratia marcescens* by addition of single amino acids, J. Bacteriol., **106**, 444–448 (1971).
54. **Cacace, J. E. and Mazza, G.:** Extraction of anthocyanins and other phenolics from black currants with sulfured water, J. Agric. Food Chem., **50**, 5939–5946 (2002).
55. **Heinemann, B., Howard, A. J., and Palocz, H. J.:** Influence of dissolved oxygen levels on production of L-asparginase and prodigiosin, Appl. Microbiol., **19**, 800–804 (1970).
56. **Solé, M., Francia, A., Rius, N., and Lorén, J. G.:** The role of pH in the 'glucose effect' on prodigiosin production by non-proliferating cells of *Serratia marcescens*, Lett. Appl. Microbiol., **25**, 81–84 (1997).
57. **Solé, M., Rius, N., Francia, A., and Lorén, J. G.:** The effect of pH on prodigiosin production by non-proliferating cells of *Serratia marcescens*, Lett. Appl. Microbiol., **19**, 341–344 (1994).
58. **Solé, M., Rius, N., and Lorén, J. G.:** Rapid extracellular acidification induced by glucose metabolism in non-proliferating cells of *Serratia marcescens*, Int. Microbiol., **3**, 39–43 (2000).

59. **Witney, F. R., Failia, M. L., and Weinberg, E. D.:** Phosphate inhibition of secondary metabolism in *Serratia marcescens*, Appl. Environ. Microbiol., **33**, 1024–1046 (1977).
60. **Rjazantseva, I. N., Andreeva, I. N., and Ogorodnikova, T. I.:** Effect of various growth conditions on pigmentation of *Serratia marcescens*, Microbios, **79**, 155–161 (1994).
61. **Yamazaki, G., Nishimura, S., Ishida, A., Kanagasabhapathy, M., Zhou, X., Nagata, S., Morohoshi, T., and Ikeda, T.:** Effect of salt stress on pigment production of *Serratia rubidaea* N-1: a potential indicator strain for screening quorum sensing inhibitors from marine microbes, J. Gen. Appl. Microbiol., **52**, 113–117 (2006).
62. **Sevcikova, B. and Kormanec, J.:** Differential production of two antibiotics of *Streptomyces coelicolor* A3(2), actinorhodin and undecylprodigiosin, upon salt stress conditions, Arch. Microbiol., **181**, 384–389 (2004).
63. **Goldschmitt, M. C. and Williams, R. P.:** Thiamin induced formation of the monopyrrole moiety of prodigiosin, J. Bacteriol., **96**, 609–616 (1968).
64. **Silverman, M. P. and Munoz, E. F.:** Effect of iron and salt on prodigiosin synthesis in *Serratia marcescens*, J. Bacteriol., **114**, 999–1006 (1973).
65. **Lawanson, A. O. and Sholeye, F. O.:** Inhibition of prodigiosin formation in *Serratia marcescens* by adenosine triphosphate, Experientia, **32**, 439–440 (1975).
66. **Li, M., Kim, T. J., Kwon, H. J., and Suh, J. W.:** Effects of extracellular ATP on the physiology of *Streptomyces coelicolor* A3(2), FEMS Microbiol. Lett., **286**, 24–31 (2008).
67. **Clements-Jewery, S.:** The reversal of glucose repressed prodigiosin production in *Serratia marcescens* by the cyclic 3′5′-adenosine monophosphate inhibitor theophylline, Experientia, **32**, 421–422 (1976).
68. **Kalivoda, E. J., Stella, N. A., Aston, M. A., Fender, J. E., Thompson, P. P., Kowalski, R. P., and Shanks, R. M.:** Cyclic AMP negatively regulates prodigiosin production by *Serratia marcescens*, Res. Microbiol., **161**, 158–167 (2010).
69. **Qadri, S. M. and Willams, R. P.:** Role of methionine in biosynthesis of prodigiosin by *Serratia marcescens*, J. Bacteriol., **116**, 1191–1198 (1973).
70. **Thomas, M. G., Burket, M. D., and Walsh, C. T.:** Conversion of L-proline to pyrrolyl-2-carboxyl-S-PCP during undecylprodigiosin and pyoluteorin biosynthesis, Chem. Biol., **9**, 171–184 (2002).
71. **Williamson, N. R., Simonsen, H. T., Ahmed, R. A. A., Goldet, G., Slater, H., Woodley, L., Leeper, F. J., and Salmond, G. P. C.:** Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*, Mol. Microbiol., **56**, 971–989 (2005).
72. **de Araújo, H. W., Fukushima, K., and Takaki, G. M.:** Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate, Molecules, **15**, 6931–6940 (2010).
73. **Harris, A. K., Williamson, N. R., Slater, H., Cox, A., Abbasi, S., Foulds, I., Simonsen, H. T., Leeper, F. J., and Salmond, G. P. C.:** The *Serratia* gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species- and strain-dependent genome context variation, Microbiology, **150**, 3547–3560 (2004).
74. **Williamson, N. R., Simonsen, H. T., Ahmed, R. A. A., Goldet, G., Slater, H., Woodley, L., Leeper, F. J., and Salmond, G. P. C.:** Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*, Mol. Microbiol., **56**, 971–989 (2005).
75. **Dauenhauer, S. A., Hull, R. A., and Williams, R. P.:** Cloning and expression in *Escherichia coli* of *Serratia marcescens* genes encoding prodigiosin biosynthesis, J. Bacteriol., **158**, 1128–1132 (1984).
76. **Williamson, N. R., Simonsen, H. T., Harris, A. K., Leeper, F. J., and Salmond, G. P.:** Disruption of the copper efflux pump (CopA) of *Serratia marcescens* ATCC 274 pleiotropically affects copper sensitivity and production of the tripyrrole secondary metabolite, prodigiosin, J. Ind. Microbiol. Biotechnol., **33**, 151–158 (2006).
77. **Yamashita, M., Nakagawa, Y., Li, H., and Matsuyama, T.:** Silica gel-dependent production of prodigiosin and serrawettins by *Serratia marcescens* in a liquid culture, Microbiol. Environ., **16**, 250–254 (2001).
78. **Tanikawa, T., Nakagawa, Y., and Matsuyama, T.:** Transcriptional downregulator hexS controlling prodigiosin and serrawettin W1 biosynthesis in *Serratia marcescens*, Microbiol. Immunol., **50**, 587–596 (2006).
79. **Kim, S. J., Lee, H. K., and Yim, J. H.:** Mutant selection of *Hahella chejuensis* KCTC 2396 and statistical optimization of medium components for prodigiosin yield-up, J. Microbiol., **46**, 183–188 (2008).
80. **Sunaga, S., Li, H., Sato, Y., Nakagawa, Y., and Matsuyama, T.:** Identification and characterization of the *pswP* gene required for the parallel production of prodigiosin and serrawettin W1 in *Serratia marcescens*, Microbiol. Immunol., **48**, 723–728 (2004).
81. **Borodina, I., Siebring, J., Zhang, J., Smith, C. P., van Keulen, G., Dijkhuizen, L., and Nielsen, J.:** Antibiotic overproduction in *Streptomyces coelicolor* A3(2) mediated by phosphofructokinase deletion, J. Biol. Chem., **283**, 25186–25199 (2008).
82. **Kang, S. G., Jin, W., Bibb, M., and Lee, K. J.:** Actinorhodin and undecylprodigiosin production in wild-type and relA mutant strains of *Streptomyces coelicolor* A3(2) grown in continuous culture, FEMS Microbiol. Lett., **168**, 221–226 (1998).
83. **Song, M. J., Bae, J., Lee, D. S., Kim, C. H., Kim, J. S., Kim, S. W., and Hong, S. I.:** Purification and characterization of prodigiosin produced by integrated bioreactor from *Serratia* sp. KH-95, J. Biosci. Bioeng., **101**, 157–161 (2006).
84. **Kim, C. H., Kim, S. W., and Hong, S. I.:** Isolation and characteristics of prodigiosin-like red pigment produced by *Serratia* sp. KH-95, Korean J. Appl. Microbiol. Biotechnol., **26**, 283–289 (1998).
85. **Kim, C. H., Kim, S. W., and Hong, S. I.:** An integrated fermentation–separation process for the production of red pigment by *Serratia* sp. KH-95, Process Biochem., **35**, 485–490 (1999).
86. **Bae, J., Moon, H., Oh, K. K., Kim, C. H., Lee, D. S., Kim, S. W., and Hong, S. I.:** A novel bioreactor with an internal adsorbent for integrated fermentation and recovery of prodigiosin-like pigment produced from *Serratia* sp. KH-95, Biotechnol. Lett., **23**, 1315–1319 (2001).
87. **Syzdek, L. D.:** Influence of *Serratia marcescens* pigmentation on cell concentrations in aerosols produced by bursting bubbles, Appl. Environ. Microbiol., **4**, 173–178 (1985).
88. **Weiss, C. M.:** Spectrophotometric and chromatographic analyses of the pigment produced by members of the genus *Serratia*, J. Cell. Comp. Physiol., **34**, 467–492 (1949).
89. **Feng, J. S., Webb, J. W., and Tsang, J. C.:** Enhancement by sodium dodecyl sulfate of pigment formation in *Serratia marcescens* O8, Appl. Environ. Microbiol., **43**, 850–853 (1982).
90. **Mallick, S. A.:** Cell surface hydrophobicity and its relation to outer membrane proteins of *Serratia marcescens*, Indian J. Exp. Biol., **134**, 107–110 (1996).
91. **Pérez-Tomás, R. and Viñas, M.:** New insights on the antitumoral properties of prodiginines, Curr. Med. Chem., **17**, 2222–2231 (2010).
92. **Yamamoto, C., Takemoto, H., Kuno, K., Yamamoto, D., Tsubura, A., Kamata, K., Hirata, H., Yamamoto, A., Kano, H., Seki, T., and Inoue, K.:** Cycloprodigiosin hydrochloride, a new H(+)/Cl(-) symporter, induces apoptosis in human and rat hepatocellular cancer cell lines in vitro and inhibits the growth of hepatocellular carcinoma xenografts in nude mice, Hepatology, **30**, 894–902 (1999).
93. **Zhang, J., Shen, Y., Liu, J., and Wei, D.:** Antimetastatic effect of prodigiosin through inhibition of tumor invasion, Biochem. Pharmacol., **69**, 407–414 (2005).
94. **Campàs, C., Dalmau, M., Montaner, B., Barragán, M., Bellosillo, B., Colomer, D., Pons, G., Pérez-Tomás, R., and Gil, J.:** Prodigiosin induces apoptosis of B and T cells from B-cell chronic lymphocytic leukemia, Leukemia, **17**, 746–750 (2003).
95. **Soto-Cerrato, V., Llagostera, E., Montaner, B., Scheffer, G. L., and Pérez-Tomás, R.:** Mitochondria-mediated apoptosis operating irrespective of multidrug resistance in breast cancer cells by the anticancer agent prodigiosin, Biochem. Pharmacol., **68**, 1345–1352 (2004).
96. **Ho, T. F., Ma, C. J., Lu, C. H., Tsai, Y. T., Wei, Y. H., Chang, JS., Lai, J. K., Cheuh, P. J., Yeh, C. T., Tang, P. C., Tsai Chang, J., Ko, J. L., Liu, F. S., Yen, H. E., and Chang, C. C.:** Undecylprodigiosin selectively induces apoptosis in human breast carcinoma cells independent of p53, Toxicol. Appl. Pharmacol., **225**, 318–328 (2007).
97. **Lagadic-Gossmann, D., Huc, L., and Lecureur, V.:** Alterations of intracellular pH homeostasis in apoptosis: origins and roles, Cell Death Differ., **11**, 953–961 (2004).
98. **Ohkuma, S., Sato, T., Okamoto, M., Matsuya, H., Arai, K., Kataoka, T., Nagai, K., and Wasserman, H. H.:** Prodigiosins uncouple lysosomal vacuolar-type ATPase through promotion of H+/Cl− symport, Biochem. J., **334**, 731–741 (1998).
99. **Yamamoto, D., Uemura, Y., Tanaka, K., Nakai, K., Yamamoto, C., Takemoto, H., Kamata, K., Hirata, H., and Hioki, K.:** Cycloprodigiosin hydrochloride, H(+)/Cl(−) symporter, induces apoptosis and differentiation in HL-60 cells, Int. J. Cancer, **88**, 121–128 (2000).
100. **Montaner, B. and Pérez-Tomás, R.:** The cytotoxic prodigiosin induces phosphorylation of p38-MAPK but not of SAPK/JNK, Toxicol. Lett., **129**, 93–98 (2002).
101. **Castillo-Avila, W., Abal, M., Robine, S., and Pérez-Tomás, R.:** Non-apoptotic concentrations of prodigiosin (H+/Cl− symporter) inhibit the acidification of lysosomes and induce cell cycle blockage in colon cancer cells, Life Sci., **78**, 121–127 (2005).
102. **Cory, S. and Adams, J. M.:** The Bcl2 family: regulators of the cellular life-or-death switch, Nat. Rev. Cancer, **2**, 647–656 (2002).
103. **Schimmer, A. D., Dalili, S., Batey, R. A., and Riedl, S. J.:** Targeting XIAP for the treatment of malignancy, Cell Death Differ., **13**, 179–188 (2006).
104. **Altieri, D. C.:** Survivin, cancer networks and pathway-directed drug discovery, Nat. Rev. Cancer, **8**, 61–70 (2008).
105. **Ho, T. F., Peng, Y. T., Chuang, S. M., Lin, S. C., Feng, B. L., Lu, C. H., Yu, W. J., Chang, J. S., and Chang, C. C.:** Prodigiosin down-regulates survivin to facilitate paclitaxel sensitization in human breast carcinoma cell lines, Toxicol. Appl. Pharmacol., **235**, 253–260 (2009).
106. **Hunter, A. M., LaCasse, E. C., and Korneluk, R. G.:** The inhibitors of apoptosis (IAPs) as cancer targets, Apoptosis, **12**, 1543–1568 (2007).
107. **Peng, X. H., Karna, P., O'Regan, R. M., Liu, X., Naithani, R., Moriarty, R. M., Wood, W. C., Lee, H. Y., and Yang, L.:** Down-regulation of inhibitor of apoptosis

proteins by deguelin selectively induces apoptosis in breast cancer cells, Mol. Pharmacol., **71**, 101–111 (2007).

108. **Menendez, D., Inga, A., and Resnick, M. A.:** The expanding universe of p53 targets, Nat. Rev. Cancer, **9**, 724–737 (2009).
109. **Manderville, R. A.:** Synthesis, proton-affinity and anti-cancer properties of the prodigiosin-group natural products, Curr. Med. Chem. Anticancer Agents, **1**, 195–218 (2001).
110. **Montaner, B., Castillo-Avila, W., Martinell, M., Ollinger, R., Aymami, J., Giralt, E., and Pérez-Tomás, R.:** DNA interaction and dual topoisomerase I and II inhibition properties of the anti-tumor drug prodigiosin, Toxicol. Sci., **85**, 870–879 (2005).
111. **Ramoneda, B. M. and Pérez-Tomás, R.:** Activation of protein kinase C for protection of cells against apoptosis induced by the immunosuppressor prodigiosin, Biochem. Pharmacol., **63**, 463–469 (2002).
112. **Soto-Cerrato, V., Viñals, F., Lambert, J. R., Kelly, J. A., and Pérez-Tomás, R.:** Prodigiosin induces the proapoptotic gene NAG-1 via glycogen synthase kinase-3beta activity in human breast cancer cells, Mol. Cancer Ther., **6**, 362–369 (2007).
113. **Songia, S., Mortellaro, A., Taverna, S., Fornasiero, C., Scheiber, E. A., Erba, E., Colotta, F., Mantovani, A., Isetta, A. M., and Golay, J.:** Characterization of the new immunosuppressive drug undecylprodigiosin in human lymphocytes: retinoblastoma protein, cyclin-dependent kinase-2, and cyclin-dependent kinase-4 as molecular targets, J. Immunol., **158**, 3987–3995 (1997).
114. **Pérez-Tomás, R. and Montaner, B.:** Effects of the proapoptotic drug prodigiosin on cell cycle-related proteins in Jurkat T cells, Histol. Histopathol., **18**, 379–385 (2003).
115. **Soto-Cerrato, V., Viñals, F., Lambert, J. R., and Pérez-Tomás, R.:** The anticancer agent prodigiosin induces p21WAF1/CIP1 expression via transforming growth factor-beta receptor pathway, Biochem. Pharmacol., **4**, 1340–1349 (2007).