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Action and function of *Chromobacterium violaceum* in health and disease: Violacein as a promising metabolite to counteract gastroenterological diseases

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ABSTRACT

Chromobacterium violaceum is a Gram negative, β-proteobacterium found in the microbiota of tropical and subtropical environments. Although considered an opportunistic pathogen, infection rapidly progress to fatal sepsis, with metastatic abscesses. It is noteworthy the multidrug resistant phenotype of *C. violaceum* and the possibility of relapse. Recently, an influence of global climate in the incidence of cases beyond the previous areas has been observed. Furthermore, chronic granulomatous disease has been considered a risk factor to infection. Despite the increase in *C. violaceum* infection incidence and high mortality, most clinicians are not familiar with it. This review pointed out important features of this life threatening microorganism, including its pathogenicity, mechanistic aspects, genetic and drug resistance associated factors, and the clinical association with chronic granulomatous disease. In addition, its main metabolite violacein may be a promising agent to counteract gastroenterological diseases, such as colorectal cancer and inflammatory gastric lesions.

Introduction

Secondary metabolites have a privileged place in microbiology since the discovery of penicillin by Fleming, which pave the way for the identification of a valuable number of medicinal compounds. It is therefore expected that tropical regions may contribute with a biodiversity with potential biotechnological and pharmaceutical applications [1]. In fact, nature still is an inexhaustible source of microbial diversity awaiting exploration, and *Chromobacterium violaceum* is one of its treasures described more than a century ago, together with its main metabolite violacein, identified as responsible for the violet colour of the bacteria colonies. In 1976, *C. violaceum* was first isolated in the borders and water of the Negro river (Amazon, Brazil), leading to the characterization of the photobiological properties of violacein, [3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one]. As a consequence of this preliminary studies and its high abundance in the Amazon region, this bacterium and its main metabolite have been studied in Brazil for the last four decades and more recently it has attracted interest from the scientific community worldwide [2–5].

Chromobacterium violaceum is a Gram-negative, rod-shaped, motile, non-fastidious, non-sporing, facultatively anaerobic, fermentative and positive for catalase and oxidase bacterium, widely distributed in the microbiota of tropical and subtropical regions. When incubated in nutrient agar, blood agar or MacConkey agar media, it produces colonies with a dark purplish colour in the pigmented strain due to its metabolite, violacein [6,7]. However, pigment production cannot be considered a trait of pathogenicity, since nonpigmented strains may have similar virulence [8–11]. Microorganism identification depends on the biochemical characterization, although detection using multiplex polymerase chain reaction targeting the prgI, spaO, invG, and sipB genes and sequencing of the 16S rDNA gene have been demonstrated [12,13].

Although this microorganism is not damaging to plants, and is

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only opportunistic to animals and humans, the infection may rapidly progress to life threatening sepsis, representing a difficult-to-treat entity. Moreover, there is a rise in the number of patients who presented with *C. violaceum* infection beyond the bacterium previous tropical and subtropical ecosystems [5,7,14]. This is of most importance since the geographic distribution of the bacterium may follow changes in global warming [7].

It is worth noting that genetic studies of *C. violaceum*, mainly conducted by the Brazilian National Genome Sequencing Consortium using the ATCC 12472 strain in 2003, revealed important characteristics of the microorganism, supporting its adaptability to the environment. In addition, these studies also provided information about the pathogenicity, metabolism, host interaction and violacein biosynthesis [15]. Accordingly, this strain was demonstrated to contain a circular chromosome of 4,751,080 bp and a G + C content of around 64,83%. Interestingly, these studies also revealed that several mechanisms contribute to *C. violaceum* coping with the plethora of stressors present in Negro river environment, such as high temperatures, lack of nutrients, high levels of radiation and toxic agents, thus justifying its abundance in this region [15]. In addition, the molecular mechanisms associated with its pathogenicity were proposed on the basis of the analysis of genes encoding possible virulence factors [15]. An important finding of this study was the presence of ORFs enconding type III secretory systems (T3SS or TTSS). T3SS is encoded by the *Chromobacterium* pathogenicity islands 1 and 1a (Cpi-1/-1a), and is involved in the translocation of proteins into the cell cytosol, where they take advantage of cellular signal transduction cascades for the pathogen benefit [16,17]. The Cpi-1/-1a-encoded T3SS is a chief inducer of cell death in a murine model of *C. violaceum* infection and in cultured mammalian cell lines, through the formation of pore structures in the host cell membrane [18]. Recently, these same authors demonstrated that CilA is a major regulator of the T3SS and they further characterized an effector protein translocated by this system, CopE (*Chromobacterium* outer protein E), which is a guanine nucleotide exchange factor (GEF) for Rac1 and Cdc42, thus playing a key role in bacterial infection of epithelial cells [17]. In addition, genes encoding factors associated with adherence and invasion process, synthesis of lipopolysaccharide (LPS) and peptidoglycan, and cytolytic proteins, such as hemolysin-like proteins, were reported [15]. Recent studies using mass spectrometry demonstrated the presence of hemolysin, collagenase, flagellar protein, metallopeptidases, outer membrane proteins, as well as the type IV secretory system (T4SS) effector protein in the culture medium of *C. violaceum* [19,20].

Pathogenicity of Chromobacterium violaceum

Despite the ample distribution of *C. violaceum* in Negro river, which is a source of drinking water for the population, this saprophyte bacterium rarely infects humans, with most cases occurring in immunocompromised individuals or children [21]. This was suggested to be related to the lack of some invasion systems currently found in other proteobacteria, such as *Salmonella typhimurium* and *Yersinia pestis* [15]. However, infections have been reported to cause skin lesions, meningitis, endocarditis, localized or metastatic abscesses, osteomyelitis, hemophagocytic syndrome, peritonitis, respiratory distress syndrome, gastrointestinal infection and sepsis, with a fatal outcome in several cases. Indeed, a rapid progression to fulminant sepsis and multiple organ dysfunction is commonly observed in *C. violaceum* infection. Urinary tract infection and diarrhea may also occur [5,7]. There is no age or gender preference and chronic granulomatous disease and glucose 6-phosphate dehydrogenase deficiency are the only conditions that seem to predispose to *C. violaceum* infection [22,23].

Other important features of the infection are its multidrug resistance and the possibility of relapse [7,25]. High mortality rates varying from 53 to 80% have been reported [7,25–27], in contrast to a more recent study of 28 patients from Australia, in which a lower mortality rate of 7.1% was observed [22]. Albeit these differences, human and animal infections with *Chromobacterium* have a major impact in public health, justifying the growing number of studies in the last few years considering the pathogenesis and treatment regimens [28–30].

The first case of *C. violaceum* infection was reported in water buffalos in the Philippines (Woolley, 1905) [31], whilst its potential pathogenicity to humans was first described in 1927, in Malaya (currently known as Malaysia) [32]. This was followed by other case reports in different countries, comprising around 150 cases with available location data worldwide, of which, 50 cases (33.3%) occurred in western Pacific (Cambodia, China, Australia, Singapore, Vietnam, Laos, Japan, Korea, Malaysia, Papua New Guinea and Solomon Islands), 2 cases (1.3%) in the Persian Gulf, 1 case (0.7%) in Europe, 46 cases (30.7%) in Americas, 29 cases (19.3%) in southeast Asia (Nepal, Thailand, India and Sri Lanka) and Africa with 22 cases (14.7%) [2,5,7,22,23,33–36]. However, these numbers must be underestimated due to the rapid evolution of the disease.

The major source of infection is through a cutaneous trauma or injury, as well as through ingestion of contaminated seafood or water. However, other routes of infection include scuba diving or near drowning, road traffic and airplane accident, and, importantly, cases of nosocomial infections have also been reported [27,34,36–40]. In this respect, Anah and colleagues [41] reported the isolation of *C. violaceum* from 10 inborn neonates. Recently, this microorganism was found in water sampled in hospitals [42,43]. The description of hospital-acquired infections warrant attention due to the life-threatening potential associated with this pathogen and the fact that most clinicians are not familiar with it.

Chromobacterium violaceum infections were recently reported in a 14-year-old boy who was diagnosed with necrotizing faciitis, in the United States [44], and in Italy, in a 14-year-old boy diagnosed with cervical lymphadenitis [13]. Recently, the number of cases in Nepal has increased [25–27,35,36]. This may be associated with progresses in laboratory diagnosis or climate change, which has led to the spread of the microorganism to other geographical locations [7]. Pant and colleagues [36] reported a case of urinary tract infection caused by *Chromobacterium violaceum* in a patient with kidney disease. In this same direction, Pant and Sharma [27] and Ma and colleagues [45], from China, have also published similar cases, whilst Swain and colleagues [46], from India, and Pant and colleagues [25] have shown urinary tract *Chromobacterium* infection in immunocompetent patients. Furthermore, a 43-year-old diabetic woman with a history of urinary catheterization and a 12-year-old girl were also diagnosed with bacteriuria associated with *C. violaceum* infection in India [34,47]. Ansari and colleagues [26] reported the first case in Nepal involving isolation of *C. violaceum* from wound sepsis that led to death due to progression to septic shock and multiorgan failure. Parajuli and colleagues [35] also recently reported a case of wound-related sepsis in a 36-year-old female; however, in this case complete recovery after antimicrobial therapy was achieved. Cases of septicemia have also been reported in India in the last few years. In only one of these cases successful treatment was achieved [48–50]. These data indicate the importance of early diagnosis of the infection and administration of proper antimicrobial treatment on the basis of susceptibility tests, even after empirical use of antimicrobial agents, to avoid the infection to progress to fatal sepsis [29].

In addition, infection has been shown to be severe in immunocompromised and malnourished patients [51]. Recently, the first case of *Chromobacterium violaceum* infection was reported in a

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severely malnourished Cambodian child [52]. Recent data have also contributed to increase the statistics of *C. violaceum* infection in Vietnam [29], Malaysia, where a fatal case of pulmonary infection was reported [53], and Taiwan, where infection was caused by a fish bite [7]. In 2015, the first case of *C. violaceum*-caused fatal bacteremia in Democratic Republic of Congo was reported in a 30-year-old man with no causal disease. In contrast, the causative pathogen was unequivocally determined in cerebrospinal fluid and blood cultures [54].

Chromobacterium violaceum infection in chronic granulomatous disease

Chronic granulomatous disease (CGD) is a heterogeneous genetic immunodeficiency that is associated with inflammatory complications and recurrent infections, including *Aspergillus* species, *Burkholderia cepacia*, *Serratia marcescens, Staphylococcus aureus*, and *Nocardia* species [55]. This disease is caused by defects in the function of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which results in impaired respiratory burst and hydrogen peroxide and superoxide production, leading to a predisposition to infection by catalase-positive organisms, such as *C. violaceum*. Mutations in any of the 5 phagocyte oxidase (*phox*) genes can lead to defects in the NADPH complex [55].

Clinical association between CGD and *C. violaceum* infection has been reported in the literature to be related to the NADPH oxidase system (NOX) [23]. Around 15% of *C. violaceum* infection was directly related to this comorbidity [7]. The majority of the cases was documented in the United States (56%) and was diagnosed with CGD after being infected with *C. violaceum* (63%) [23]. Most of the patients presented severe disease, with several cases of septicemia, multiple abscesses in skin and soft-tissue, lymphadenitis and one case of fascial necrotizing fasciitis and another one of suppurative jugular thrombophlebitis [23]. From the sixteen cases reported to date, only two patients died [51,56]. However, the incidence of *C. violaceum* infection in CGD patients must be underestimated. As most patients succumbed to death, the diagnostic test for CGD may not have had done or it may not have been available, since it is confirmed by nitroblue tetrazolium reduction or dihydrorhodamine oxidation test of neutrophil function and gene sequencing of NADPH mutations [22,23,55]. Thus, it is recommended to test for neutrophil function those young patients with *C. violaceum* infection as this bacterium may be an indicator of CGD disease.

Recently, Maltez and colleagues [57] proposed an interesting role of inflammasomes in the clearance of *C. violaceum* infection by inducing pyroptosis and natural killer cell cytotoxicity. The inflammasome is a macromolecular complex involved in innate immunity to control cytosolic pathogenic microorganisms and danger signals. It is formed by the inflammasome-initiating sensors (NLRP1 [nucleotide-binding oligomerization domain (NOD) leucine-rich repeat (LRR)-containing protein (NLR)], NLRP3, NLRC4, pyrin or AIM2 [absent in melanoma 2]) and inflammatory caspases, in the absence or presence of the inflammasome adapter protein ASC. Activation of inflammasome-associated caspases induces the cleavage of the pro-pyroptotic factor, gasdermin D, which oligomerizes and drives the production of cytokines and cell demise by pyroptosis [58]. Inflammasomes can be activated by caspase-1 dependent (canonical) and caspase-1 independent mechanisms (non-canonical). Caspase-1 activation promotes the process and the release of interleukin-1β (IL-1β) and IL-18, and to the activation of pyroptosis, whereas the non-canonical pathway is executed by human caspases-4 and 5, and mouse caspase-11 [58]. The NAIP-NLRC4 inflammasome was initially identified to act in response to bacterial flagellin. Further studies also demonstrated that NLRC4 responds to bacterial T3SS by detecting specifically the bacterial proteins flagellin, T3SS rod and T3SS needle, when they translocated to the cytosol by using the NLR family, apoptosis inhibitory proteins (NAIPs) as upstreams receptors [59]. However, in humans, only one NAIP is found, which is responsible for recognizing the T3SS needle subunit of *C. violaceum* [60,61], although an extended isoform of NAIP from macrophages was reported to respond to *Salmonella* flagellin [62].

Studies using inflammasome-deficient mice have concluded that inflammasomes slow the infectious process until an adaptive immune response eradicates infection. However, when engineered *S. typhimurium* expressing flagellin was used to infect inflammasome-deficient mice, it remained virulent, whereas it was completely attenuated in wild-type mice by a mechanism involving pyroptosis and neutrophil production of reactive oxygen species (ROS) [59]. This led the authors to hypothesize that there are environmental microbes that act in the same way as the engineered evasion-deficient *Salmonella*, which would almost never cause infection in immunocompetent individuals due to the inflammasome detection and activation of innate immunity [57].

Chronic granulomatous disease is characterized by a number of mutations in NOX and, thus it may present low levels of ROS derived from phagocytes. Susceptibility of patients with CGD to different intracellular bacteria, such as *C. violaceum*, was proposed to be dependent on its ability to eject bacteria from the intracellular compartment by pyroptosis [57]. Indeed, *C. violaceum* was shown to be highly virulent in *Ncf1*$^{-/-}$ mice (encodes p47^{phox}) [63] and in *Casp1*$^{-/-}$*Casp11*$^{-/-}$ mice [57]. In addition, an extensive neutrophilic infiltrate and macroscopic lesions in the livers of these mice were observed, which are in agreement with the liver tropism of *Chromobacterium* in CGD [7,57]. This study also pointed out NLRC4-driven pyroptosis as a major mechanism to protect the spleen, where macrophages are abundant. Furthermore, the release of IL-18 in the liver activated NK cell cytotoxicity [57]. In conclusion, inflammasomes direct sterilizing innate immunity against lethal infection with *C. violaceum*.

Treatment of *C. violaceum* infection in CGD patients has followed the same protocols described for non-CGD patients, with gentamicin, chroramphenicol fluoroquinolones, trimethoprim/sulfamethoxazole and carbapenems included in the therapeutic regimens reported so far [7,22,23,51,56].

Drug resistance in Chromobacterium violaceum

Due to the still limited number of cases, results of antimicrobial susceptibility testing vary among different clinical settings. In this respect, most strains of *C. violaceum* are resistant to penicillins and beta-lactam antibiotics, such as cephalosporin, due to its increased levels of beta-lactamase [7,24]. Also, this bacterium has shown resistance to rifampin and vancomycin, which makes the treatment difficult [28]. On the other hand, susceptibility to chloramphenicol, trimethoprim, sulfamethoxazole, tetracyclines, ciprofloxacin, cefepime and imipenem has been demonstrated. Thus, the broad spectrum antibiotics, such as carbapenems or fluoroquinolones, can be used as appropriate choices for control *Chromobacterium* infection considering that therapeutic guidelines are not available. Gentamicin and amikacin are of intermediate susceptibility [7,28]. Prolonged treatment is recommended as a high frequency of hematogenous dissemination to visceral organs and relapse is observed [7,24].

In a comprehensive study, Fantinatti-Gaboggini and colleagues [24] described gene sequences in *C. violaceum* ATCC 12472 associated with drug resistance. Besides the genes encoding beta-lactamases, efflux systems acting as unidirectional pumping transporters of cytotoxic drugs from the cell to the extracellular

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media is a key mechanism of multiple drug resistance in bacteria. Five classes of drug effux translocases have been described: small multidrug resistance (SMR), ATP binding cassette (ABC), major facilitator (MF), multidrug and toxic compound extrusion (MATE), and resistance nodulation cell division (RND). The genome of *C. violaceum* was characterized by the presence of genes similar to the multiple antibiotic resistance (*mar*) locus, whose *mar*RAB operon is known to control resistance in *Escherichia coli*. An ORF with similarity to *mar*C is present in *C. violaceum* [24].

Genes with similarity with the multidrug resistance pumps of the MF and SMR families are present in *C. violaceum*. The former efflux pumps protect bacteria from several unrelated antibiotics. For instance, the bicyclomycin resistance protein (bcr) characteristic of the MF family was identified in *C. violaceum*. The translocase EmrE of the SMR family is a small protein that confers resistance to tetracycline, erythromycin, and sulfadiazine. A gene similar to *emr*E is present in *C. violaceum*. Also, several genes with similarity to the ATP-binding transport system and to the RND family of efflux system, which mediate resistance to the macrolide acriflavine, were described. Overexpression of other gene products, such as the MdfA, results in resistance to puromycin, rifampicin, tetracycline, chloramphenicol and erythromycin. A homolog of this gene was also identified in *C. violaceum* [24]. These results indicate that antibiotic resistance is an important factor to be overcome in the clinic of *C. violaceum* infection.

Application of *C. violaceum* secondary metabolite, violacein, in anticancer therapy

A number of studies in the literature have pinpointed the biological potential and clinical significance of the main metabolite of *C. violaceum* characterized to date, known as violacein. Despite the research involving violacein activites in the defense mechanisms of the bacteria against eukaryotic predation and fungal disease, this purple metabolite has attracted more interest for its potential pharmacological activities, including antitumoral, antibacterial, antiprotozoal, antiviral, antioxidant, antifungal, leishmanicidal and trypanocidal activities, most of them reviewed recently [5]. In contrast to the most studied activities of violacein, literature involving its immunomodulatory potential is still scarce and it has only emerged after 2010, with the publication of its role in inflammatory and adaptive responses of a few animal models, including delayed type hypersensibility, anaphylactic reaction, pyrexia, and autoimmune encephalomyelitis (EAE) [5,64,65]. In this respect, violacein was shown to also demonstrated the possibility to control EAE using adoptive transfer of violacein-elicited regulatory T (Treg) cells, which was associated with the induction of Treg cells and the regulation of dendritic cells and inflammatory modulators, such as IL-10, IL-17 and indoleamine 2,3-dioxygenase [64].

Of note in the field of gastropathies, Antonisamy and colleagues [66] reported that violacein promoted a gatroprotective effect in an indomethacin-induced gastric ulcer rat model. Moreover, this activity was K^+ATP-channel dependent. Further evaluation of the mechanisms involved in this activity revelead that violacein upregulated the mucosal levels of prostaglandin E_2 (PGE_2). Pretreatment with SC560 indicated that this effect is mediated through cyclooxygenase 1 (COX1). In addition, violacein reduced the proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-1β and IL-6, while increasing the anti-inflammatory cytokines (IL-4 and IL-10) and growth factors (vascular endothelial growth factor - VEGF, endothelial growth factor - EGF, and hepatocyte growth factor - HGF) compared with the indomethacin-ulcerated group. These factors have important roles in modulating ulcer healing by increasing angiogenesis and gastric mucin, while decreasing gastric acid secretion. Violacein restored the normal levels of constitutive nitric oxide synthase (cNOS) in the mucosa, which pointed to a role for NO in gastroprotection. Violacein also inhibited apoptosis probably as a consequence of the decrease in TNF-α, a cytokine involved in cell death receptor activation [66]. These results highlight the development of this violet secondary metabolite as an antiulcer agent.

The antitumor potential of violacein is perhaps the most studied feature of this metabolite and over the last few years, research have advanced in our knowledge of its action, highlighting its development as an anticancer agent. Studies in this area started with the demonstration of its cytotoxicity in V79 fibroblasts using the tetrazolium salt reduction, the nucleic acid content and the uptake of the neutral red dye methodologies. In addition, this was the first time that violacein was demonstrated to induce apoptosis [67]. At this time, violacein was also reported to inhibit the growth of a number of cancer cell lines, showing major results against NCI-H460 non-small-cell lung cancer and KM12 colon cancer cell lines and MOLT-4 leukemia, with GI_{50} (the concentration that inhibits cell growth by 50%) ranging from 0.03 to 0.06 μM [67]. These studies instigate the exploration of its potential antitumor effects in several tumor cells, as well as the investigation of its mechanisms of action. In this regard, major efforts have been devoted to unravel the mechanisms of violacein-induced cell death.

Interestingly, violacein has been shown to display lineage-specific cell death characteristics and mode of action. Previous studies demonstrated violacein-induced apoptosis in the myeloid leukemia cell line, HL60, in its free form and complexed to β-cyclodextrin [68]. This was followed by the demonstration that this effect was mediated by the tumor necrosis factor receptor (TNFR) signaling pathway, leading to caspase-8 and p38 mitogen-activated protein kinase (p38 MAPK) activation, and nuclear factor κB (NFκB) translocation [69]. Activation of TNFR1 was specific to HL60 cells, since this effect was not observed in U937 and K562 leukemias, as well as in normal human lymphocytes, which present a higher IC_{50} (50% inhibitory effect on cellular viability) value (10 μM) compared with HL60 cells (<1.0 μM) [69,70]. Recently, violacein activity was evaluated in the resistant TF1 leukemia cells, demonstrating for the first time a non-canonical mechanism of cell death [71]. Violacein clearly induced TF1 cell death, with an IC_{50} of 2 μM. Notably, the results also demonstrated the absence of direct necrosis and apoptosis despite the increased nuclear fragmentation, which is in accordance with the increase in poly ADP-ribose polymerase (PARP) cleavage. Furthermore, violacein-induced TF1 cell death depicted a 'horseshoe-shaped' nucleus, as well as endoplasmic reticulum and Golgi linearization, resulting in organelle collapse and cell demise. Noteworthy, kinome profiling experiments indicated that this effect was mediated by the inhibition of death-associated protein kinase 1 (DAPK1) and calpain, and the activation of Akt, PKA and PDK [71]. These promising results in a resistant $CD34^+$/c-Kit^+/P-$glycoprotein^+$/$MRP1^+$ cancer cell line open new opportunities to evaluate the effect of this violet metabolite in other malignancies expressing similar features, such as gastrointestinal stromal tumors (GIST), which express de KIT (c-kit) receptor tyrosine kinase, and in most cases are resistant to chemotherapy due to the expression of high levels of P-glycoprotein and MRP1, as well as the mutations in KIT and PDGFRA [72].

Of major importance is the ability of violacein to counteract colorectal cancer. Colorectal cancer (CRC) is a major cause of morbidity and mortality around the world, affecting 1.36 million people globally, being considered the second most common cancer in women and the third most common cancer in men [73,74]. Although the introduction of effective screening programs has led to a reduction in the incidence of CRC in some regions, in others, lifestyle and poor infrastructure conditions have contributed to an increase in CRC [74,75]. In addition, in recent years the progress in

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cancer biology has turned into therapeutic advances in the treatment of gastrointestinal malignancies. However, most therapies are still based in chemotherapeutic agents and patients still die, especially due to cancer resistance and relapse. Thus, it is important to seek for new effective anticancer drugs. In this respect, bioactive compounds, such as violacein, which is able to counteract CRC, have an important role for envisage the development of new drugs. Indeed, Kodach and colleagues [76] reported that violacein have cytotoxic properties in colon cancer cell lines, such as Caco-2, HCT 116, SW480, DLD1 and HT29, with IC_{50} values ranging from 1.5 mM to >10 mM [76]. These authors have shown the ability of this secondary metabolite to reestablish the chemosensitivity and cause apoptosis in the 5-fluorouracil-resistant HCT 116 cell line through a mechanism that involves activation of Akt and downregulation of NFκB. Violacein also blocked G1 transition in these cells by inducing p21, p53 and p27, whereas it decreased the levels of cyclin D1 [76]. These results are of most importance since interfering selectively in survival signaling cascades by the synergistic combination of two agents, violacein and 5-FU, can be an approach to reactivate the apoptotic pathway in resistant CRC. In addition to these results, de Carvalho and colleagues [77] also demonstrate the ability of violacein to induce apoptosis by elevating the levels of oxidative stress in Caco-2 colon cancer cells, but not in HT29 cells [77], further underscoring the existence of cell type-specific mechanisms targeted by violacein. Furthermore, in Caco-2 cells the release of calcium and cytochrome *c* into the cytoplasm play a role in the activation of caspase 3 [77]. Based on these results one can envisage future research to establish violacein potential in in vivo models of gastrointestinal malignancies.

The role of oxidative stress in violacein activation of apoptosis was also corroborated in the Ehrlich ascites tumor (EAT) model [78]. Moreover, this was the first study to demonstrate the in vivo antitumor activity of violacein. Doses of 0.1 and 1.0 μg/kg administered intraperitoneally (i.p.) resulted in increased lifespan of tumor-bearing mice and significant inhibition of tumor volume. Most importantly, this was the first consistent in vivo study of violacein toxicity, showing data for complete biochemistry, hematology, and histopathological analysis of kidney and liver [78].

In recent years the antitumor potential of violacein has been explored in different cancer cell lines as model systems, including glioblastoma (U87) [79], melanoma (SKMEL-103 and SKMEL-28) [80], breast (MCF7) [79,81–83], lung (A549) [79], cervical (HeLa) [84], and head and neck carcinoma (HN5, SCC-15; CAL-27, FaDu, SALTO) [82,85], offering new opportunities to explore its mechanisms of action.

Leal and colleagues [79] explored the relationship between cell death and ROS in HeLa cervical cancer cell line exposed to violacein. The authors also investigated cytotoxicity in other two non-tumorigenic cell lines, the CHO-K1 (Chinese hamster ovary cells) and MRC-5 (human fetal lung fibroblasts), and they concluded that it occurred primarily by necrosis, whereas cytotoxicity to HeLa was mediated mainly by apoptosis. Contrary to the effects observed in EAT [73] and colon cancer cells [72], no link between increased oxidative stress and cell death was found. In addition, violacein-induced HeLa and MRC-5 cell death was specifically associated with the hyperpolarization of the mitochondrial membrane [79].

Platt and colleagues [74] investigated violacein cytotoxicity to MCF7 breast cancer cells, aiming to elucidate its activity on invasion and metastasis. Metastasis requires metalloproteinases (MMP) to degradate the extracellular matrix, which causes the invasion of the membrane basement and inflammation [81]. In addition, cell adhesion and migration are stimulated by inflammatory chemokines, thus affecting the metastatic process. In breast cancer cells, a non-cytotoxic concentration of violacein (1 μM) downregulated the CXCL12/CXCR4 interaction [76]. This chemokine axis has been associated with the pathogenesis of leukemias, breast and pancreatic cancers, as is involved in the migration of metastasis-initiating cancer cells [82]. Moreover, knockdown of MMP-2 showed that in the presence of violacein there was an inhibition of MMP-2-mediated secretion of CXCL12. In addition, in co-cultures violacein abrogated the upregulation of MMP-9 activity. Together, these results indicate that this indole derivative could inhibit the first and late stages of inflammation by acting on MMP-2 expression and activation, respectively, as well as on CXCL12/CXCR4 interaction [76]. The CXCL12/CXCR axis plays important roles in controlling the interaction between the tumor cell and its microenvironment as well as in angiogenesis, thus open new possibilities for violacein to develop new anticancer therapies by acting on the tumor cell microenvironment.

Alshatwi and colleagues [78] explored the time and dose-dependent mechanism of action of violacein in MCF7 breast cancer cells. Violacein inhibited cell viability in a time and dose-dependent manner, showing an IC_{50} value of 1.7 μM after 48 h of treatment. Significant ROS production was observed even at low concentrations of violacein (0.25 μM). Treatment at all time points induced morphological signs of apoptosis and evaluation of the expression profile of apoptotic genes induced by violacein revealed upregulation of Bax, p53, caspase-8, caspase-3, Fas, Fas-associated death domain (FADD), TNF-α and PARP, whilst it downregulated Bcl-2 and MDM2, suggesting involvement of the intrinsic and extrinsic pathways of apoptosis [78].

Recently, Mehta and colleagues [74] have studied violacein activity and mode of action in lung (A549), breast (MCF7) and glioblastoma (U87) cancer cell lines. Since an increased incidence of brain metastasis is often associated with lung and breast cancers, the authors included glioblastoma cells in the study, aiming to assess the ability of violacein to inhibit migration. Proliferation of all cancer cell lines was inhibited by violacein and a greater sensitivity of lung and glioblastoma cells to the compound was observed in viability assays (IC_{50} = ~1 μM). Analysis of the mechanisms of action indicated activation of ERK1/2 and PARP in U87 cell death. It is important to note that violacein also inhibited migration of glioblastoma cells due to its capacity to disrupt the actin filamentous network, including lamellipodia and filopodia, leading to a round cellular morphology that compromised motility [74]. These results further contribute to previous study showing the inhibitory effect of violacein on cancer metastatic invasion [76].

In a recent interesting study, violacein exhibited cytotoxicity against RAF and RAS-mutated metastatic melanoma cells, with an IC_{50} value around 0.5 μM. Importantly, this was the first time that violacein was shown to inhibit autophagy in melanoma cells and to diminish histone deacetylase 6 (HDAC6) expression, leading to a drop in cell proliferation and an induction of cell death by apoptosis. In addition, violacein significantly inhibited invasion of metastatic melanoma in 3D spheroid cultures [75]. This research shed new light on the intriguing mechanism of violacein-induced cell death.

In a work published by Masuelli and colleagues [80], evidence for the ability of violacein to prevent in vitro and in vivo head and neck cancer cell growth was provided. In vitro, the study demonstrated that violacein increased the Bax/Bcl-2 ratio and cleaved PARP, while it diminished the activation of ERK1/2 and the expression of LC3-II. This indole derivative was also shown to induce p53 degradation and the production of ROS, as well as to inhibit NFκB translocation. Of major importance, this study demonstrated that intratumoral administration of 0.75 mg/kg violacein delayed the growth of SALTO cancer cells and increased the median survival of tumor-bearing animals [80].

In an interesting work published by Hashimi and colleagues [77], the antitumor effect of violacein was evaluated under hypoxia

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in several cell lines, including MCF7, HCT 116, HT29 and HN5. Results indicated a considerable improvement in violacein activity, with significant reduction of the IC_{50} values, varying from 4 fold (MCF7) to 12.6 fold (HT29). Furthermore, this purple derivative exhibited in vivo efficacy in a HN5 subcutaneous model tumor growth [77].

Taken together, these studies, including those in vivo, bring new evidence of violacein anticancer potential even in resistant and aggressive cancer cells. They also draw attention to the need to increase research to unravel the mechanisms involved in its activity. In this respect, recent investigation of the mechanisms of the antibacterial activity of violacein in *S. aureus* ATCC 29231 and methicillin-resistant *S. aureus* ATCC 43300 (MRSA) add new knowledge to our understanding of violacein interaction with cells. This work revealed that this derivative causes disruption of membrane integrity, mesosome formation, and leaking of intracellular constituents in both bacterial strains, suggesting biological membranes as possible targets of violacein [83]. Moreover, collectively, these studies highlight the status of microbial secondary metabolites in research, challenging the development of new natural-based chemotherapeutic agents.

Summary

Whilst *C. violaceum* is a rare entity, infection with this pathogen may become emergent after climate change. Thus, clinicians must be aware of its diagnosis and optimal antimicrobial therapy. Moreover, further studies will provide additional clues on the mechanisms involved and risks of infection. Secondary metabolites from natural sources have increased the human life span during the last centuries, helping to revolutionize the practice of medicine. Microbes have provided leads to the development of a number of compounds that had a major impact in the fate of several diseases. *C. violaceum* is one of such microorganisms found in a tropical ecosystem, which is able to produce a number of secondary metabolites of interest, such as violacein, FK901228, and deoxyviolacein, some of which are still unexplored. In the last few years, we have witnessed an increased interest in the biological properties and applications of its purplish-colored pigment, violacein, including in gastroenterological diseases with potential results. It is unquestionable that violacein has become a notorious biological tool for application in a diverse range of biotechnological, industrial and pharmacological fields. This is suggested by the significant number of patents and considering the perspectives in all these areas still not entirely explored, it is conceivable to foresee an increase in the number of publications in the near future.

Practice points

- *C. violaceum* infection has a major impact in public health due to the high frequency of hematogenous dissemination to visceral organs and relapse, the high mortality rate and its multidrug resistant phenotype.
- Malnourished and immunocompromised individuals, such as patients with chronic granulomatous disease, are predisposed to *C. violaceum* infection.
- Violacein is a promissor biological tool in anticancer therapy, the most studied feature of this metabolite, including the gastrointestinal malignancies.

Research agenda

- *C. violaceum* infection may become emergent after climate change.
- The mechanisms associated with *C. violaceum* infection needs to be further defined.
- Therapeutic guidelines based on antimicrobial susceptibility tests need to be defined.
- Further studies are necessary to clarify violacein in vivo activities and its toxicological profile.
- The role of violacein in *C. violaceum* needs to be defined.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Justo GZ, Souza ACS, de Fátima A, Pedrosa MFF, Ferreira CV, Rocha HAO. The medicinal value of biodiversity: new hits to fight cancer. In: Grillo O, Venora G, editors. Biological diversity and sustainable resources use. Rijeka: InTech; 2011. p. 121–58.
[2] Durán N, Justo GZ, Ferreira CV, Melo PS, Cordi L, Martins D. Violacein: properties and biological activities. Biotechnol Appl Biochem 2007;48:127–33.
[3] Durán M, Faljoni-Alario A, Durán N. *Chromobacterium violaceum* and its important metabolites - review. Folia Microbiol 2010;55:535–47.
[4] Durán M, Ponezi AN, Faljoni-Alario A, Teixeira MFS, Justo GZ, Durán N. Potential applications of violacein: a microbial pigment. Med Chem Res 2012;21:1524–32.
[5]* Durán N, Justo GZ, Durán M, et al. Advances in Chromobacterium violaceum and properties of violacein - its main secondary metabolite: a review. Biotechnol Adv 2016;34:1030–45.
[6] Durán N, Menck CFM. *Chromobacterium violaceum*: a review of pharmacological and industrial perspectives. Crit Rev Microbiol 2001;27:201–22.
[7]* Yang CH, Li YH. *Chromobacterium violaceum* infection: a clinical review of an important but neglected infection. J Chin Med Assoc 2011;74:435–41.
[8] Miller DP, Blevins WT, Steele DB, Stowers MD. A comparative study of virulent and avirulent strains of *Chromobacterium violaceum*. Can J Microbiol 1988;34:249–55.
[9] Sivendra R, Lo HS. Identification of Chromobacterium violaceum: pigmented and non-pigmented strains. J Gen Microbiol 1975;90:21–31.
[10] Díaz Peres JA, García J, Rodriguez Villamizar LA. Sepsis by *Chromobacterium violaceum*: first case report from Colombia. Braz J Infec Dis 2007;11:441–2.
[11] Guevara A, Salomón M, Oliveros M, Guevara E, Guevara M, Medina Z. Sepsis por *Chromobacterium violaceum* pigmentado y no pigmentado. Rev Chil Infect 2007;24:402–6.
[12] Scholz HC, Witte A, Tomaso H, Al Dahouk S, Neubauer H. Detection of Chromobacterium violaceum by multiplex PCR targeting the prgI, spaO, invG, and sipB genes. Syst Appl Microbiol 2006;29:45–8.
[13] Arosio M, Raglio A, Ruggeri M, et al. *Chromobacterium violaceum* lymphadenitis successfully treated in a Northern Italian hospital. New Microbiol 2011;34:429–32.
[14] Ponte R, Jenkins SG. Fatal *Chromobacterium violaceum* infections associated with exposure to stagnant waters. Pediatr Infect Dis J 1992;11:583–6.
[15]* Brazilian National Genome Project Consortium. The complete genome sequence of Chromobacterium violaceum reveals remarkable and exploitable bacterial adaptability. Proc Natl Acad Sci U. S. A 2003;100:11660–5.
[16] Galán JE, Wolf-Watz H. Protein delivery into eukaryotic cells by type III secretion machines. Nature 2006;444:567–73.
[17]* Miki T, Akiba K, Iguchi M, Danbara H, Okada N. The *Chromobacterium violaceum* type III effector CopE, a guanine nucleotide exchange factor for Rac1 and Cdc42, is involved in bacterial invasion of epithelial cells and pathogenesis. Mol Microbiol 2011;80:1186–203.
[18]* Miki T, Iguchi M, Akiba K, et al. Chromobacterium pathogenicity island 1 type III secretion system is a major virulence determinant for Chromobacterium violaceum-induced cell death in hepatocytes. Mol Microbiol 2010;77:855–72.
[19] Ciprandi A, da Silva WM, Valadares Santos AV, et al. *Chromobacterium*

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violaceum: important insights for virulence and biotechnological potential by exoproteomic studies. Curr Microbiol 2013;67:100–6.
[20] Castro-Gomes T, Cardoso MS, Da Rocha WD, et al. Identification of secreted virulence factors of *Chromobacterium violaceum*. J Microbiol 2014;52:350–3.
[21] Richard C. Chromobacterium violaceum, opportunist pathogenic bacteria in tropical and subtropical regions. Bull Soc Pathol Exot 1993;86:169–73.
[22] Lin Y, Majumdar SS, Hennessy J, Baird RW. The spectrum of *Chromobacterium violaceum* infections from a single geographic location. Am J Trop Med Hyg 2016;94:710–6.
[23]* Meher-Homji Z, Mangalore RP, D R Johnson P, Y L Chua K. Chromobacterium violaceum infection in chronic granulomatous disease: a case report and review of the literature. JMM Case Rep 2017;4:e005084.
[24]* Aldridge KE, Valainis GT, Sanders CV. Comparison of the in vitro activity of ciprofloxacin and 24 other antimicrobial agents against clinical strains of *Chromobacterium violaceum*. Diagn Microbiol Infect Dis 1988;10:31–9.
[25] Byamukama D, Farnleitner AH, Kansiime F, Manafi M, Burtscher M, Mach RL. Contrasting occurrence of Chromobacterium violaceum in tropical drinking water springs of Uganda. J Water Health 2005;3:229–38.
[26] Ansari S, Paudel P, Gautam K, Shrestha S, Thapa S, Gautam R. Chromobacterium violaceum isolated from a wound sepsis: a case study from Nepal. Case Rep Infect Dis 2015;2015:181946.
[27] Pant ND, Sharma M. Urinary tract infection caused by Chromobacterium violaceum. Int J Gen Med 2015;8:293–5.
[28]* Fantinatti-Garboggini F, Almeida Rd, Portillo Vdo A, et al. Drug resistance in Chromobacterium violaceum. Genet Mol Res 2004;3:134–47.
[29] Campbell JI, Lan NP, Qui PT, Dung le T, Farrar JJ, Baker S. A successful antimicrobial regime for Chromobacterium violaceum induced bacteremia. BMC Infect Dis 2013;13:4.
[30] Richard KR, Lovvorn JJ, Oliver SE, Ross SA, Benner KW, Kong MY. Chromobacterium violaceum sepsis: rethinking conventional therapy to improve outcome. Am J Case Rep 2015;16:740–4.
[31] Woolley PG. Bacillus violaceus manilae (a pathogenic microorganism). Bull Johns Hopkins Hosp 1905;16:S9–93.
[32] Sneath PH, Whelan JP, Bhagwan Singh R, Edwards D. Fatal infection by *Chromobacterium violaceum*. Lancet 1953;265:276–7.
[33] Jitmuang A. Human *Chromobacterium violaceum* infection in Southeast Asia: case reports and literature review. Southeast Asian J Trop Med Public Health 2008;39:452–60.
[34] Kaniyarakkal V, Orvankundil S, Lalitha SK, Thazhethekandi R, Thottathil J. Chromobacterium violaceum septicaemia and urinary tract infection: case Reports from a tertiary care hospital in South India. Case Rep Infect Dis 2016;2016:6795743.
[35] Parajuli NP, Bhetwal A, Ghimire S, et al. Bacteremia caused by a rare pathogen - Chromobacterium violaceum: a case report from Nepal. Int J Gen Med 2016;9:441–6.
[36] Pant ND, Acharya SP, Bhandari R, Yadav UN, Saru DB, Sharma M. Bacteremia and urinary tract infection caused by *Chromobacterium violaceum*: case reports from a tertiary care hospital in Kathmandu, Nepal. Case Rep Med 2017;2017:7929671.
[37] Lee J, Kim JS, Nahm CH, et al. Two cases of *Chromobacterium violaceum* infection after injury in a subtropical region. J Clin Microbiol 1999;37:2068–70.
[38] Victorica B, Baer H, Ayoub EM. Successful treatment of systemic *Chromobacterium violaceum* infection. JAMA 1974;230:578–80.
[39] Starr AJ, Cribbett LS, Poklepovic J, Friedman H, Ruffolo EH. *Chromobacterium violaceum* presenting as a surgical emergency. South Med J 1981;74:1137–9.
[40] Chen CH, Lin LC, Liu CE, Young TG. Chromobacterium violaceum bacteremia: a case report. J Microbiol Immunol Infect 2003;36:141–4.
[41] Anah MU, Udo JJ, Ochigbo SO, Abia-Bassey LN. Neonatal septicaemia in Calabar, Nigeria. Trop Doct 2008;38:126–8.
[42] Umadevi S, Kumar S, Stephen S, Joseph NM. Chromobacterium violaceum: a potential nosocomial pathogen. Am J Infect Control 2013;41:386.
[43] Hagiya H, Murase T, Suzuki M, et al. *Chromobacterium violaceum* nosocomial pneumonia in two Japanese patients at an intensive care unit. J Infect Chemother 2014;20:139–42.
[44] Seigel JK, Stadler ME, Lombrano JL, Almony JS, Couch ME, Belhorn TH. *Chromobacterium violaceum* necrotizing fasciitis: a case report and review of the literature. Ear Nose Throat J 2012;91:479–83.
[45] Ma T, Shi W, Cheng J, et al. *Chromobacterium violaceum* infection in China: Three case reports and literature reviews. Afr J Microbiol Res 2011;5:3096.
[46] Swain B, Otta S, Sahu KK, Panda K, Rout S. Urinary tract infection by *Chromobacterium violaceum*. J Clin Diagn Res 2014;8:DD01–2.
[47] Mohan V, Rajan R, Haneefa S. *Chromobacterium violaceum* causing urinary tract infection: a case report. J Acad Clin Microbiol 2014;16:90–3.
[48] Karthik R, Pancharatnam P, Balaji V. Fatal *Chromobacterium violaceum* septicemia in a south indian adult. J Infect Devel Ctries 2012;6:751–5.
[49] Kar H, Mane V, Urhekar AD, et al. A first case report in tertiary care hospital, Navi Mumbai, India *Chromobacterium violaceum* septicaemia in a child. Int J Curr Microbiol Appl Sci 2013;2:245–9.
[50] Madi DR, Vidyalakshmi K, Ramapuram J, Shetty AK. Successful treatment of Chromobacterium violaceum sepsis in a south indian adult. Am J Trop Med Hyg 2015;93:1066–7.
[51] Rashid Z, Ali U, Sulong A, Rahman R. *Chromobacterium violaceum* infections; a series of case reports in a Malaysian tertiary hospital. Internet J Infect Dis 2013;11:1.
[52] Ke L, An KP, Heng S, et al. Paediatric *Chromobacterium violaceum* in Cambodia: the first documented case. Trop Doct 2012;42:178–9.
[53] Cheong BMK. A fatal case of pulmonary *Chromobacterium violaceum* infection in an adult. Med J Malays 2010;65:148–9.
[54] Bottieau E, Mukendi D, Kalo JR, et al. Fatal Chromobacterium violaceum bacteraemia in rural Bandundu, democratic republic of the Congo. New Microbes New Infect 2014;3:21–3.
[55] Marciano BE, Spalding C, Fitzgerald A, et al. Common severe infections in chronic granulomatous disease. Clin Infect Dis 2015;60:1176–83.
[56] Sirinavin S, Techasaensiri C, Benjaponpitak S, Pornkul R, Vorachit M. Invasive *Chromobacterium violaceum* infection in children: case report and review. Pediatr Infect Dis J 2005;24:559–61.
[57]* Maltez VI, Tubbs AL, Cook KD, et al. Inflammasomes coordinate pyroptosis and natural killer cell cytotoxicity to clear infection by a ubiquitous environmental bacterium. Immunity 2015;43:987–97.
[58] Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol 2016;16(7):407–20.
[59] Miao EA, Mao DP, Yudkovsky N, et al. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. Proc Natl Acad Sci U. S. A 2010;107:3076–80.
[60] Zhao Y, Yang J, Shi J, et al. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature 2011;477:596–600.
[61] Yang J, Zhao Y, Shi J, Shao F. Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. Proc Natl Acad Sci U. S. A 2013;110:14408–13.
[62] Kortmann J, Brubaker SW, Monack DM. Cutting Edge: inflammasome activation in primary human macrophages is dependent on flagellin. J Immunol 2015;195:815–9.
[63]* Segal BH, Ding L, Holland SM. Phagocyte NADPH oxidase, but not inducible nitric oxide synthase, is essential for early control of Burkholderia cepacia and Chromobacterium violaceum infection in mice. Infect Immun 2003;71:205–10.
[64] Verinaud L, Lopes SC, Prado IC, et al. Violacein treatment modulates acute and chronic inflammation through the suppression of cytokine production and induction of regulatory T cells. PLoS One 2015;10:e0125409.
[65] Rosenblum MG, Chen X. Multimodality molecular imaging with therapeutic conjugates. US Patent 2010;20100068151 A1.
[66]* Antonisamy P, Kannan P, Aravinthan A, et al. Gastroprotective activity of violacein isolated from *Chromobacterium violaceum* on indomethacin-induced gastric lesions in rats: investigation of potential mechanisms of action. Sci World J 2014;2014:616432.
[67] Melo PS, Maria SS, Vidal BC, Haun M, Durán N. Violacein cytotoxicity and induction of apoptosis in V79 cells. In Vitro Cell Dev Biol Anim 2000;36:539–43.
[68] Melo PS, Justo GZ, de Azevedo MB, Durán N, Haun M. Violacein and its beta-cyclodextrin complexes induce apoptosis and differentiation in HL60 cells. Toxicology 2003;186:217–25.
[69] Ferreira CV, Bos CL, Versteeg HH, Justo GZ, Durán N, Peppelenbosch MP. Molecular mechanism of violacein-mediated human leukemia cell death. Blood 2004;104:1459–64.
[70] Bromberg N, Justo GZ, Haun M, Durán N, Ferreira CV. Violacein cytotoxicity on human blood lymphocytes and effect on phosphatases. J Enzyme Inhib Med Chem 2005;20:449–54.
[71] Queiroz KC, Milani R, Ruela-de-Sousa RR, et al. Violacein induces death of resistant leukaemia cells via kinome reprogramming, endoplasmic reticulum stress and Golgi apparatus collapse. PLoS One 2012;7:e45362.
[72] Kang W, Zhu C, Yu J, Ye X, Ma Z. KIT gene mutations in gastrointestinal stromal tumor. Front Biosci (Landmark Ed) 2015;20:919–26.
[73] Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2016;66(2016):7–30.
[74] Siegel R, Desantis C, Jemal A. Colorectal cancerstatistics. CA Cancer J Clin 2014;64(2014):104–17.
[75] Issa IA, Noureddine M. Colorectal cancer screening: An updated review of theavailable options. World J Gastroenterol 2017;23:5086–96.
[76]* Kodach LL, Bos CL, Durán N, Peppelenbosch MP, Ferreira CV, Hardwick JC. Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells. Carcinogenesis 2006;27:508–16.
[77]* de Carvalho DD, Costa FT, Duran N, Haun M. Cytotoxic activity of violacein in human colon cancer cells. Toxicol Vitro 2006;20:1514–21.
[78] Bromberg N, Dreyfuss JL, Regatieri CV, et al. Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumor. Chem Biol Interact 2010;186:43–52.
[79] Mehta T, Vercruysse K, Johnson T, Ejiofor AO, Myles E, Quick QA. Violacein induces p44/42 mitogen-activated protein kinase-mediated solid tumor cell death and inhibits tumor cell migration. Mol Med Rep 2015;12:1443–8.
[80] Gonçalves PR, Rocha-Brito KJ, Fernandes MR, Abrantes JL, Durán N, Ferreira-Halder CV. Violacein induces death of RAS-mutated metastatic melanoma by impairing autophagy process. Tumour Biol 2016;37(10):14049–58.
[81] Platt D, Amara S, Mehta T, et al. Violacein inhibits matrix metalloproteinase mediated CXCR4 expression: potential anti-tumor effect in cancer invasion and metastasis. Biochem Biophys Res Commun 2014;455:107–12.
[82] Hashimi SM, Xu T, Wei MQ. Violacein anticancer activity is enhanced under hypoxia. Oncol Rep 2015;33:1731–6.
[83] Alshatwi AA, Subash-Babu P, Antonisamy P. Violacein induces apoptosis in

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human breast cancer cells through up regulation of BAX, p53 and down regulation of MDM2. Exp Toxicol Pathol 2016;68:89–97.

[84] Leal AM, de Queiroz JD, de Medeiros SR, Lima TK, Agnez-Lima LF. Violacein induces cell death by triggering mitochondrial membrane hyperpolarization in vitro. BMC Microbiol 2015;15:115.

[85] Masuelli L, Pantanella F, La Regina G, et al. Violacein, an indole-derived purple-colored natural pigment produced by *Janthinobacterium lividum*, inhibits the growth of head and neck carcinoma cell lines both in vitro and in vivo. Tumour Biol 2016;37:3705–17.

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