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Review Article

Breast Cancer and Cell Cycle Inhibitors (CCIs): Potential Therapeutic Strategies for CCI Cell Targeting and Drug Delivery

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Abstract

Cancer is a genetic instability disorder caused by the accumulation of successive gene mutations. Breast cancer (BC) and its metastases are the most common cause of cancer death in young and older women worldwide. It is well known that abnormalities in the positive and negative modulators of the cell (growth) cycle occur frequently in many cancers including Breast Carcinomas (BC). Recent advancements in clinical studies have been reported regarding BC patient's survival times using a class of heterocyclic drugs termed Cell Cycle Inhibitors (CCIs). Such third generation FDA-approved CCI synthetic drugs are cyclin-dependent kinase 4/6 inhibitors, which act by inhibiting progression of the G1-to-S phase transition of the cell cycle. However, deleterious side effects of these drugs can affect five main areas of patient well-being, namely; 1) bone marrow depletion; 2) gastrointestinal distress; 3) increased risk for infections; 4) cardiac wave interval delays; and 5) CCI drug resistance. In view of reports of increased BC patient survival times, seeking new and novel therapeutic options for CCIs gains new importance. In order to achieve improved patient care and survival, the potential use of antimicrobial-like peptides is addressed in the present report regarding cancer cell targeting, plasma membrane penetration, and intracellular drug delivery.

Keywords: Breast cancer; Cell cycle; Cell survival; Drug delivery; Growth arrest; Inhibitors; Peptides Targeting

Abbreviations:

AMPL-Antimicrobial like peptide

APC - tumor suppressor gene

ATm = ataxia telangectasia mulated

BRCA - breast cancer related gene

CDK - cyclin dependent kinase

CHK - Kinase checkpoint proteins

FANCA - Fanconi anemia cancer related gene

HECt - Homologous to E6-associated protein-C terminus

MCM - DNA licensing replication factor

SKP2, 1 - F-Box protein regulation of G1-S phase

Ring protein - ubiquitin RING domain

Introduction

Cancer is a chromosomal/genetic instability disorder caused by an accumulative series of DNA mutations in the cells and tissues of the body [1]. As a consequence, the main properties exhibited by cancer cells are uncontrolled cell division, growth, and proliferation. The unstable genome of malignant cells is comprised of aberrant mitosis and growth pathways involving regulatory networks in cell cycle progression, phase checkpoints, and DNA damage sensing and repair systems [2]. Normally, DNA damage would be restored and repaired during cell cycle progressive phase transitions; however, mutated and defective protein members of the DNA damage-sensing and repair networks and the CCIs allow the passage of corrupted DNA through the cell cycle transitions leading to aberrant cell mitosis, replication, and growth.

The mechanism that provides the cell the means to divide, grow, and proliferate is an intrinsic clock-like series

of regulatory coordinated steps collectively referred to as the cell cycle; this process leads to mitotic cell division. The functional objective of the cell cycle process is to prepare the cell to divide by synthesizing new DNA for subsequent replication and growth [3]. The activities of this built-in cell growth clock are achieved through four transition phase changes, namely, the G1, S, G2, and M [mitosis] phases. The cycling clock is roughly 12-24 hours in duration depending on the specific cell type and species. In order to assist and regulate the phase transitions, five different classes or types of proteins are required. These proteins consist of: a] cyclins; b] Cyclin-Dependent Kinases [CDK] and their inhibitors; c] checkpoint passage regulatory proteins; d] DNA damage sensing/repair proteins; and e] proteasomal ubiquitin degrading agents [4]. Cyclins are proteins that enhance cell cycle progression by activating and binding to CDKs. Cyclin-dependent kinases are serine/threonine kinase enzymes that form complexes with various cyclins to phosphorylate protein substrates required for cell cycle progression [5, 6]. Checkpoint phase transition and DNA damage/repair moieties are proteins that can either promote or arrest cell cycle progression depending on the damage status of the DNA. Ubiquitins are proteasomal derived-agents that degrade cell cycle constituent proteins by binding, ligating, and catalyzing the breakdown of these proteins [7]. The cell cycle inhibitors, such as p21 and p27, bind to the cyclins and block cyclin/CDK complexation [interaction], thus preventing further cell cycle progression. If the CCIs are degraded by the ubiquitin system, cyclins are free to complex with the various CDKs and engage in subsequent cell cycle progression [8]. The absence and/or dysfunction of CCIs in cancer cells promote the unregulated growth of the tumors. Hence, adding and/or administering synthetic CCIs to breast cancer patients serve to block further tumor growth and increase patient survival times [9].

Overall, the presence of non-mutated natural CCIs serve to halt the G1-to-S phase progression of the cell cycle as needed. There are several means by which the cell cycle can be arrested and halted. First, synthetic CCIs can be administered to cancer patients by oral ingestion or other means in order to boost or replace the loss of total CCIs in patients' cancer cells. Secondly, drugs could be added or injected into cancer cells to bind cyclins and/or CDKs in order to prevent Cyclin/CDK complexing interaction and subsequent cell cycle progression. Third, an agent could be injected into breast cancer patients which has the capability to down regulate the RNA tissue/cell expression of either cyclins, CDKs, or proteasomal ubiquitins. Down-regulation of the cyclins, for example, would decrease the number of cyclins that could bind CDKs; this step would promote cell cycle arrest at the G1-to-S transition phase [10].

Objectives and aims

The objectives of the present report are several in number. First, the cell cycle progression and transition procedures are reviewed for the less-informed reader. Second, the recently developed synthetic cell cycle inhibitor drugs [heterocyclic hydrocarbons] are discussed, mainly the third-generation drugs, regarding their growth inhibitory efficacy and multiple patient side effects. Third, alternate therapeutic drug options in place of CCIs will be presented concerning growth inhibition, cell targeting, and drug delivery. Fourth, the provision of companion drug modalities, such as the use of peptides and their modified mimics, are presented which could provide other/additional chemotherapeutic options to arrest or disable cell cycle progressing phase transition. Although peptides and/or their mimetics could serve to complement use of heterocyclic drugs in chemotherapy, such peptides could also be utilized alone to deliver conjugated chemo-drugs into cancer cells.

Naturally-occurring cell cycle inhibitors

Naturally-occurring Cell Cyclin Inhibitors [CCIs] constitute one of the many positive and/or negative modulators of the cell cycle within both normal and malignant cells [i.e., breast cancer]. Cancer cell mutation abnormalities, such as defective functions of the retinoblastoma and p53 gene and the Cyclin-Dependent Kinases [CDKs], are often observed in breast and other cancers [11]. CCIs involved in these activities encompass proteins such as p16 [INK4], p21 [CIP/WAF2], and the p27 [KIP1] inhibitors all of which halt, arrest, or slow cell cycle transitions at the G1-to-S phase transition [12]. CCIs block the ability of cyclins to bind the CDKs and thereby prevent the CDK phosphorylating activation process. The individual synthetic CCIs are addressed in Section IV.

The first of the CCIs, p16 [INK4], binds to Cyclin-D and blocks CDK4 from complexing with this cyclin; this prevents phosphorylation of the retinoblastoma [Rb] protein [13]. INK4 dysregulation is found in tumors of the lung, pancreas, melanomas, and nasopharynx tissues. The second of the CCIs, p21 [CIP/WAF1], complexes with several different cyclins and prevents CDK1, CDK2, CDK4, and CDK6 interactions [14]. The p21 [CIPWAF1] affects p53 activity and is significantly correlated with lymph node metastases. The third of the CCIs, p27 [KIP1], binds and interacts with Cyclin-E, Cyclin-D, and Cyclin-A blocking the activation of CDK1, CDK2, CDK4, and CDK6 [15]. The KIP1 protein blocks the phosphorylation of Rb protein and IGF signaling in tumors of the breast, prostate, pancreas, colon, and lung. Dysregulation of the various activation pathways, such as the Rb gene, can occur by multiple mechanisms such as gene amplification or rearrangement, loss of regulators, epigenetic alterations, and point mutations in key pathway components [14].

The synthetic cell cycle inhibitor drugs

As discussed above, the cell cycle components govern the cells transition from quiescence through mitosis, cell growth, and eventually to cell proliferation. In reproductive cancers such as breast and uterus, cyclins are often upregulated while CDKs and CCIs are largely rendered defective and dysfunctional [16]. The use of synthetic CCI drugs have advanced to include greater CDK specificity in third generation drugs, such as Ribociclin, which have

gained clinical prominence in cancer patient survival [9]. These synthetic CCIs are used in combination therapy with the estrogen-synthesis [aromatase] inhibitors such as letrozole. Recent reports, utilizing ER[+]/HER [-]BC patients administered CCIs, showed increased survival times from 30% to 60%, together with mortality rates at 30% or less and lower drug toxicity [9].

At present, three synthetic CCIs have been FDA-approved for ER[+]/HER[-] BC in postmenopausal women. Such CCIs include: a] palbociclib; b] ribociclib; and c] abemociclib; a fourth CCI, triaciclib, remains investigational [17]. All four CCIs are CDK4/6 inhibitors are utilized in patients with hematologic, neuroendocrine, endometrial, breast, and liposarcomas cancers. More recently, ribociclib has shown utility in treating metastatic BC when combined with letrozole and fulvestrant [9]. Although CDK4/6 inhibitors are the most commonly utilized CCIs in the clinic, the potential use of other CDK and transcription factor inhibitors remain. Although differences among the FDA-approved CCIs occur regarding toxicities, dosing schedules, patient monitoring techniques, and side effects, most drugs appear to delay cancer progression and increase patient survival times [18-20]. Most dosing schedules of synthetic CCIs employ 28-30day duration for at least 6 treatment cycles or more. Overall, the side effects of synthetic CCI drugs do not differ much from the standard chemotherapeutic drugs presently in use [see below].

Side effect in patients receiving CCIs

Adverse side effects have been reported in patients receiving third generation [CDK- specific] CCI drugs in oral dosages employing multiple cycles. Using Ribociclib [RCL] as the example, this CCI produces many and varied deleterious side effects in 60 to 80% of patients [9, 11]. RCL treatment causes bone marrow suppression and anemia resulting in lowered white blood cells levels [neutropenia] together with decreased red blood cell counts and thrombocytopenia. The patients are at increased risk for infection, display alopecia, and produce gastrointestinal distress such as nausea, vomiting, diarrhea, and loss of appetite. Other side effects reported encompass biochemical and physiological issues such as prolonged cardiac QT intervals requiring mandatory EKG measurements, elevated liver transaminase enzyme blood levels, and development of CCI drug resistance. Furthermore, RCL accumulates in the body with a half-life of 32 hours and is eliminated via the feces and urine. Because RCL is degraded in the liver by the cytochrome P450 enzyme CYP3A4, this enzyme and its spinoff CCI metabolites increase in the bloodstream with the potential to cross-react and inhibit other drugs taken by the patient; in addition, these products compete for binding to other transporter blood proteins. Although RCL binds to circulating plasma proteins, the synthetic CCI drug is not specifically and selectively targeted into cancer cells [see below]. In addition, the cost-to-patient monthly fee is $12,000.00 prior to insurance company arbitration settlements which can reduce patient costs. Finally, synthetic CCIs weaken the patient's immune system via reduction in white blood cell levels causing an increased susceptibility to infection, inflammation, and slowed wound healing.

Alternative therapeutic strategy options

Cancer Cell Targeting and Drug Delivery: Presently, there exists a need for patients receiving synthetic chemodrugs for more specific cancer cell targeting in order to reduce deleterious side effects in patients. Although CCIs have produced dose-dependent BC growth inhibition, satisfactory efficacy, and improved patient survival, these promising results come with a high cost of debilitating side effects [17]. Even though synthetic CCIs are highly selective for inhibiting certain subsets of CDKs in breast and other cancers, they consist of heterocyclic benzo-nitrogenous hydrocarbons which lack a means to specifically target to cancer cells (Table-1).

CCIs are transported in blood by carrier proteins which are non-directed, non-specific, and non-selective for their cargo [drug] transport into normal or cancer cells. The carrier blood proteins which bind CCIs are comprised of either serum albumin and/or α_2 macroglobulin with only 25-30% uptake and carrier capabilities [23-25]. Ideally, one should logically pursue new and novel therapeutic agents capable of either: a] specific cancer cell targeting while retaining CCI activities; or b] employing a cancer cell targeting agent conjugated to a CCI drug as its cargo. Although the options of choices "a and b" may be one and the same agent, it could also encompass two different compounds. Naturally-occurring and synthetic peptides such as antimicrobial-like [AMPL] peptides possess multiple properties which could aid as an agent in these goals. Such properties of AMPLs include: 1] excellent cancer cell targeting and uptake capabilities; 2] interference with signaling transduction pathways; 3] production of receptor blockade; 4] service as decoy binding ligands for receptors; and 5] boosting immune system function in cancer patients [26, 27-31]. Peptide administration and/or injections of AMPLs into patients could potentially include agents such as free peptides, peptides conjugated to drugs, or peptide mimetics.

The poor cancer cell targeting of heterocyclic drugs has remained a clinical challenge since the post-World War II discovery of chemo drugs; however, this obstacle remains at present. Multiple vehicles for patient drug delivery to cancer cells are varied and numerous. These might include oral pills, muscle/intravenous injections, sublingual liquid droplets, electroporation, lipofection, nanoparticles, and dendrimer injections. Regardless of the mode of administration of heterocyclic CCIs into the human body, only a third or less actually reaches the cancer mass for cell entry. Much of the administered drug is taken up by the reticuloendothelial cells, a system of phagocytic scavenger cells lining the body's blood vascular and lymphatic vessels. As the blood transport protein [albumin] bearing the hydrocarbon drug approaches the cancer cell mass, the carrier protein cannot distinguish cancer cells from non-malignant cells. Thus, the transporting blood protein delivers the bound drug indiscriminately into

both cancer and non-malignant normal cells. Furthermore, the blood carrier protein cannot directly penetrate the cancer cell membrane in order to deliver the drug into the cell cytoplasm.

The drug-bearing blood carrier protein, such as albumin, does not have the capability to specifically home to a cancer cell instead of a normal cell. This fact largely contributes to the deleterious side effects ascribed to these drugs in the preceding sections. The drug-transporting blood protein is capable of eventually gaining entry into the cancer or normal cell by non-specific means; such proteins have been found to enter cells by endocytosis assisted by co-transcytoses with other carrier blood protein such as the SPARC protein [29]. After cell entry, the blood proteins [and cargo] are ferried to the lysosome compartment for degradation and their cargo may or may not be released into the cytoplasm. In contrast, AMPL peptides, following uptake, are vesicle-bound and transported directly to the perinuclear compartment of the cell [31]. Moreover, drugs covalently conjugated to a carrier blood protein are not easily dislodged from the protein because covalent bonds are often formed from the amino acid lysine, which is a strong binding agent. One such solution to achieve both specific cells targeting and drug delivery is the use of one of either two distinct classes of peptides i.e., 1] the short cell penetrating peptides [CPPs], and the b] longer antimicrobial peptides [AMPs] [26,27]. The biomedical literature is replete with reports of naturally-occurring protein-derived peptide fragments and synthetic peptide counterparts with both cells penetrating and drug delivery capabilities [27-30].

One such example of a microbial-like peptide is the growth inhibitory peptide [GIP] fragment derived from full length alpha-fetoprotein [AFP], a tumor-associated fetal protein [27]. The AFP-derived 34-mer peptide fragment lies buried in a molecular cleft of the tertiary-folded protein [30]. When exposed following a conformational transformation, the intrinsic 34-amino acid sequence section of human AFP temporarily converts the growth-enhancing full-length molecule into a growth inhibitory protein [31]. The transformed growth inhibiting AFP molecule present during pregnancy can temporarily halt growth until signal pathways can be repaired and restored in the fetus. The transformed AFP molecule then refolds into its tertiary native configuration again concealing the GIP segment. The 34-amino acid GIP and its subsegments have been synthesized as a free peptide and isolated, purified, and their biological activities characterized [32]. The GIP fragment has been reported to inhibit growth in breast and other human cancers in both in vivo and in vitro studies [30]. Interestingly, the GIP fragment demonstrates many of the properties displayed by antimicrobial-like [AMPL] peptides, especially those of cell targeting, penetration, and cell cytoplasmic entry. The GIP fragment is an amphoteric peptide, similar to naturally-occurring AMPs consisting of multiple cationic, hydrophobic, and zwitterionic amino acids culminating in a largely positive-charged molecule. AMP-like peptides can penetrate [bore] into a cell membrane via a non-receptor-mediated mechanism [26].

Drug delivery into targeted cancer cells: It is of interest that microbes such as bacteria, fungi, enveloped viruses, and transformed cancer cells display an overall net negative cell surface charge [34, 35]. The negative surface charge on microbes and cancer cells is attributed to a phospholipid inversion [phosphoglycerol/phosphatidylserine event] in the outermost leaflet of the bilayer plasma membrane of cancer cells Lipid inversion is a well-established cell membrane event in which the negatively-charged polar heads of inner leaflet membrane phospholipids invert [flip] and emerge to the outer leaflet surface layer of the cancer cell membrane [36]. It is known that normal cells display a net cell surface positive charge in contrast to the negatively-charged cancer cells. It is the net cell surface charge that distinguishes transformed cancer cells from non-malignant normal cells. Similar to a homing device, AMPL-peptides can seek out and electrostatically attach to cancer cells. These peptides can then penetrate the cell membrane and enter into the cytoplasmic compartment [26,27]. Thus, AMPL-peptides have the capability to specifically target to cancer cell but not normal calls.

Therapeutic options employing drug carrying peptides: Concerning the delivery of drug cargos into cancer cells, the cell penetrating peptide and/or the AMPs can be conjugated to chemo-drugs for intracytoplasmic drug delivery [26,38,39]. In fact, certain cargos can be bound to peptides via a covalent bond or attach in a non-covalent binding [affinity] fashion. It is noteworthy that peptides can be conjugated to heterocyclic drugs which can be delivered and gain entry into cancer cells. In a published in vitro report, an 8-mer sub-fragment of GIP conjugated to Doxorubicin [DOX] proved to be more effective in cancer growth inhibition than DOX alone [33]. Thus, AMP-Like peptides such as GIP can selectively target cancer cells, penetrate, and deliver drugs into multiple cancer cell types including BC. Some AMP-Like peptides, such as GIP, can not only penetrate the bilayer cell membrane but can further inhibit breast cancer growth by causing dysregulation of the cell cycle. The 34-mer GIP itself need not carry a drug cargo [payload] in order to suppress cancer growth since two of its mechanisms employ both RNA downregulation and cell membrane pore-forming/channel blocking procedures each of these block cell cycle progressions.

Both of the above mechanisms have been studied by: 1] RNA microarray analysis, and 2] Sharp's electrode and patch-clamp electrophysiology measurements [40]. In the electrophysiological studies, the 34 mer GIP was demonstrated to exhibit both cell membrane pore forming and channel blocking abilities. In comparison, the 8-mer GIP subsegment was largely found to block cell membrane channel blocking/interaction activities [40]. It is now well-established that channel interacting peptides are directly linked by signal pathways to cell cycle G1-to-S phase transition [41, 42]. In both instances, the two GIP peptides were found to decrease and/or stabilize the cell membrane potential at physiologic peptide molar concentrations and gain entrance into the BC cell interior. In an international

Table 1: A Heterocyclic Cell Cycle Inhibitory [CCI] Drug and an Antimicrobial-like Peptide are Listed and Compared According to their Biological and Biochemical Characteristics, Traits, and Properties. Examples used are Ribociclib [CCI] and Growth Inhibitory Peptide [AMP-like].

Characteristics, Traits, and Properties	Heterocyclic CCI Inhibitor Drug [Ribociclib]	Antimicrobial-Like Peptide [Growth Inhibitory Peptide]	References Cited
1]. Cancer Growth and Proliferation	Inhibits growth of Breast, Endometrial, Ovarian and Prostate Cancers	Inhibits growth of Breast, Ovary, Kidney, Prostate, Lymphoma Cancers	9,22,19,29,31
2] Cell Cycle phase [transition] progression	Blocks cell cycle progression at G1-to-S phase transition	Blocks cell cycle progression at G1-to-S phase transition	12, 13
3] Cyclin specificity and interaction	Specific only for Cyclin-D	Specific for Cyclin-D and E; interacts with Cyclins-A, B, C, G, H	1, 4, 6, 11
4] Cyclin-dependent kinase [CDK] specificity and interaction	Specific only for CDK4 and CDK6	Specific for CDK4 and 6; interactions with CDK1, 2, 5, 7	12, 14, 20
5]Cell cycle associated [CCA] protein interaction	No reported interaction	Binding and/or interaction with CHK1, CHK2, CDKAK1, CDKI3, MCM-2, 3, 4, 5	1, 4, 11
6] Proteosomal ubiquitin ligase interaction	No reported interaction	Binding and/or interaction with Cull 1, 2, 3; WD40 SKP2, APC, SKP1, UBIQ-E1; HECT,	1, 4, 6
7] DNA-Damage Sensing and Repair proteins	No reported interaction	Binding and/or interaction with BRCA1, BRCA2, FANC1, DNA-Kinases ATm, ATR/RAD3	1, 4, 6
8] Specific cancer cell targeting	Incapable of specific targeting to cancer cells and normal cells	Amphoteric nature targets only to negative-charged cancer cells, not positive- charged normal cells	23, 24, 26
9] Cell membrane penetration	No reported capability	Amphoteric [AMP-Like] peptides form cell membrane pores and/or channels	26, 27, 36
10] Cell membrane transpassage [internalization]; cell entry	CCIs transported by blood carrier proteins; undergo endocytosis and assisted transcytosis	AMP-like peptides permealize into outer leaflets of Bi-layer cell membrane	26, 27, 36
Characteristics, Traits, and Properties	Heterocyclic CCI Inhibitor Drug [Ribociclib]	Antimicrobial-Like Peptide [Growth Inhibitory Peptide]	References Cited
11] Cargo delivery into cancer cells	No reported capability	AMP-like peptides can be either conjugated to drugs or naturally bound to them	38, 39
12] Cytokine interactions	No reported interactions	AMP-L pro-inflammatory cytokine induction; induces nitric oxide synthase, PGE2 production	28
13] Effect on Host Immune response	Treatment produces risk of infection in patient	AMPLs promotes and enhances the immune response of patients	26, 27
14] Side effects on Host or patient	Bone marrow depletion, neutropenia, thrombocytopenia, G.I. Distress, and cardiac interval lag effect	AMPls produce little if any side effect in animal models; possible immunogenic and allergic effects	9, 13

collaborative study, it was further reported that both 34-mer GIP and 8-mer GIP inhibited growth in breast, ovarian, melanoma, and lung cancers in cell culture as well as in human breast cancer- mouse xenografts models *in-vivo* [33]. In fact, using mammary tumor models in mice, it was demonstrated that radioactive I^{125} labeled 34-mer peptide localized 3 times greater in the tumor mass than remained in blood at 24 hours post-injection [43].

Alternative means to inhibit BC growth and halt cell cycle progression: The heterocyclic CCIs have been reported to inhibit BC growth and halt cell cycle progression. [9,14]. In comparison, the inhibition of cancer growth via cell cycle arrest by GIP can also be achieved by RNA down-regulation of synthesized cell cycle proteins such as ubiquitins [SUMO sentrin, RING ligands] which degrade the natural CCIs. In addition, RNA down-regulation of the cyclins and CDK- RNA transcripts can occur that arrests cell cycle progression [i.e., cyclins, SKP2, and check point regulators] [33] (Table 1). It would be quite reasonable to predict that RNA down-regulation of even a few of the cell cycle-associated proteins could influence the function of multiple cell cycle proteins affecting cell cycle progression.

In addition to downregulating RNA transcripts that promote protein synthesis, other AFP derived peptides were demonstrated to bind additional Cell Cycle-Associated [CCA] proteins [1,4,6]. These CCA proteins included CDK-related proteins, check point regulators, and various proteasomal ubiquitins. In comparison, the RNA downregulations and CCA protein binding to AFP-derived peptides cannot be duplicated by the heterocyclic CCI drugs currently in clinical use [9,13,14] (Table.1). Furthermore, the heterocyclic drugs display no specific cell targeting, and cell membrane penetration properties (Table.1). Such characteristics of AMPL peptides would be beneficial for blocking cell cycle phase transitions.

Patient side effects of CCI drugs versus AMPL peptides: The deleterious side effects of heterocyclic CCIs in BC patients have been addressed in Section-V of this report (Table 1). Such side effects can be summarized and grouped into five

major effects, namely, 1] bone marrow cell depletions; 2] risk of infection; 3] gastrointestinal distress; 4] cardiac interval wave delays; and 5] CCI drug resistance [15]. In contrast to the CCI drugs, AMPs and AMP-like peptides have long been utilized in man and animals to combat microbial infection, boost host cell immunity, display anti-inflammatory activities, and enhance wound healing. The safety records of AMP use in patients have been impressive showing little or marginal side effects. No significant toxicological changes have been observed in hundreds of animal models that received high doses [200 mg/Kg] of AMPs [30]. Unfortunately, comparable clinical data of AMP use in human cancer patients have yet to be determined and reported. No animal deaths or abnormalities were recorded in preclinical studies using criteria such as body weights, food consumption, urinalysis, hematology, and blood chemistry analyses. All rodent organ weights [liver spleen, heart, kidneys, and intestines] were normal compared to controls [44, 45]. Most importantly, AMPL peptides did not produce multi-drug resistance since many AMPs are naturally-occurring peptides derived directly from natural full-length proteins [27] The lack of side effects were also found in mice tested for toxicity following multiple GIP injection [200 mg/mouse] at the National Cancer Institute [NCI] at Fort Dietrich in Frederick, Maryland. [Author's personal communication]. During that same testing period, the NCI also reported that GIP-34 tested in multiple cancer cell cultures inhibited 38 of 60 different cancer cell lines assayed *in vitro* [30, 31, 45, 46].

Advantages and disadvantages in the Use of AMP-like peptides: It is important to also address both the advantages and disadvantages of the use of AMPs as potential chemotherapeutic agents. The past clinical use of AMPs was intended to by-pass the multi-drug resistance developed to microbial pathogens produced by over-use of present-day antibiotics [48]. Overall, AMPs and AMP-like peptides display excellent cell targeting and drug delivery properties, have few serious side effects, exhibit short half-lives, and good bioavailability. Although not previously reported, AMPs have yet to show some immunogenic and allergic side effects, and demonstrate less than optimal physical and/or chemical properties [49]. Due to the high cost of large-scale production, pharmaceutical companies have been reluctant to pursue AMP-like peptide production and development. However, such commercial production problems can be overcome by the falling costs of synthetic peptide analogs and the heterologous production of recombinant peptides. In the future, AMPs could possibly be employed for the rational design of peptide mimetics to overcome the drawbacks found in some naturally-occurring therapeutic peptides. Recently, AMPs have caught the attention as alternative antibiotic and anticancer agents due to their primary structure containing Beta-hairpin loops stabilized by disulfide bridges [50]. Overall, the emerging goals in the future use of AMPL peptides would be to avoid and/or bypass the adverse side effects of present-day chemotherapies, all while boosting the immune system, aiding in immune modulation and wound healing, and reducing inflammation in the cancer patient [51].

Conclusion

The growth cycle of cells plays a major role in DNA syntheses and in cell preparation and progression for mitotic cell division, replication, growth, and proliferation. The cyclin-dependent kinases, such CDK4/6 and CDK2, are the pivotal drivers of cell growth in combination with Cyclin-D and Cyclin-E, respectively. These Cyclin/CDK formed complexes normally serve to enhance cell cycle progression from the G1-to-S phase transition of the cell cycle. However, the naturally-occurring CCIs, such as p21 CIP and p27 KIP, are susceptible to mutational alterations leading to defective function of the retinoblastoma and p53 gene products often observed in breast cancers. It was the presence of dysfunctional natural CCIs that led to the pharmacological development of synthetic heterocyclic CCIs [palbociclib, ribociclib, abemaciclib], especially the CDK-specific third generation of such drugs. As noted above, these synthetic CCIs have demonstrated notable BC growth inhibition and improved survival times in patients. Unfortunately, the synthetic CCIs produce a significant array of deleterious side effects.

In view of the above clinical disadvantages in using CCIs, the present report has strived to provide potential new and novel therapeutic options for the clinical chemo drug use in BC patients. One such therapeutic approach would involve the utilization of antimicrobial-like peptides [AMPs] and/or peptide mimetics to overcome the above adverse side effects, drawbacks, and disadvantages attributed to synthetic CCI drugs. The AMPs have clinically been employed to boost host immunity, bypass antibiotic drug resistance, reduce inflammation to microbial infections, and more recently poised for use as anticancer agents. As discussed above, AMP-like peptides demonstrate few if any side effects in preclinical human cell culture and animal studies, and show excellent cell targeting and drug delivery properties.

In summation, one could propose that the ideal therapeutic strategy for BC patients might be to combine the advantages of both the heterocyclic CCI drugs and the AMPL peptides (Table 1). Hypothetically, this could entail either one or both of two possibilities, namely; a] separate injections [administrations] of a CCI and of an AMPL peptide into the same patient; and/or b] injection of a heterocyclic CCI drug conjugated to an AMPL peptide into a patient. These procedures may represent the "best of both worlds" of cancer chemotherapeutic strategies.

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