Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2021, 12, 337-359
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Dermaseptin B2's Anti-Proliferative Activity and down Regulation of Anti-Proliferative, Angiogenic and Metastatic Genes in Rhabdomyosarcoma RD Cells *in Vitro*

Ahmed A. Abdille[1*], Josephine Kimani[2], Fred Wamunyokoli[1,2], Wallace Bulimo[3], Yahaya Gavamukulya[4], Esther N. Maina[1,5]

[1]Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences Technology and Innovation (PAUSTI), Nairobi, Kenya
[2]Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
[3]Centre for Public Health Research, Kenya Medical Research Institute, Nairobi, Kenya
[4]Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, Mbale, Uganda
[5]Department of Biochemistry, College of Health Sciences, University of Nairobi, Nairobi, Kenya
Email: *ahmedabbi7@gmail.com

How to cite this paper: Abdille, A.A., Kimani, J., Wamunyokoli, F., Bulimo, W., Gavamukulya, Y. and Maina, E.N. (2021) Dermaseptin B2's Anti-Proliferative Activity and down Regulation of Anti-Proliferative, Angiogenic and Metastatic Genes in Rhabdomyosarcoma RD Cells *in Vitro*. *Advances in Bioscience and Biotechnology*, **12**, 337-359.
https://doi.org/10.4236/abb.2021.1210022

Received: August 29, 2021
Accepted: October 25, 2021
Published: October 28, 2021

Abstract

Background: Rhabdomyosarcoma (RMS) is the most prevalent soft tissue sarcoma in children, representing approximately 50% of pediatric sarcomas and can develop in any part of the body though more frequently at the extremities. **Aim:** Evaluating the *in vitro* anti-proliferative activity of Dermaseptin B2 on Rhabdomyosarcoma RD (CCL-136TM) cells and its effect on the expression of *MYC, FGFR1, NOTCH1*, and CXCR7 genes involve in processes including proliferation, angiogenesis and metastasis. **Methods:** RD cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% Fetal Bovine Serum. Exponentially growing cells were treated with Dermaseptin B2 and Antiproliferative activity was assayed using the resazurin and migration assays at three time-points. In order to determine the gene expression profiles of *MYC, NOTCH1, FGFR1* and *CXCR7*, total RNA was extracted from the cells and q-RT-PCR was performed with *β-Actin* as reference gene. **Results:** Dermaseptin B2 inhibited the proliferation of RD cells in a time and concentration dependent manner as with IC_{50} values of 7.679 µM, 7.235 µM, 5.993 µM. The 2-dimentional wound healing assay showed inhibition of migration and motility of the RD cells at time-points of 6, 24, 48 and 72-hours with the greatest inhibition observed at 72-hours. Dermaseptin B2 downregulated the target *MYC* (fc; 1.5013, 1.5185, 2.4144), *CXCR7* (fc; 2.8818, 4.4430, 3.9924),

FGFR1 (fc; 2.3515, 2.0809, 2.2543), *NOTCH1* (fc; 2.4667, 4.6274, 4.3352) genes for the three-time points respectively. *NOTCH1* and *CXCR7* showed higher fold changes with respect to *β-Actin* than *MYC* and *FGFR1*. **Conclusion:** The results of this study indicate that Dermaseptin B2 is a target molecule for signaling pathways including PI3K/AKT, RTK and NOTCH pathways that could affect the transcription of these genes and overall inhibition of cancer progression. Further studies are needed to give a better understanding of the detailed mechanisms of action as well as the effects of the Dermaseptin B2 peptide *in vivo.*

Keywords

Dermaseptin B2, Doxorubicin, RMS, Angiogenesis, Metastasis

1. Introduction

Rhabdomyosarcoma (RMS) is the most prevalent soft tissue sarcoma in children [1] [2], representing approximately 50% of pediatric sarcomas [1] [3] [4], which can develop in any part of the body though more frequently at the extremities [5]. Rhabdomyosarcoma is an aggressive tumor that classically originates from skeletal muscle progenitor cells [3] [6] and represents 4% - 5% of the childhood cancers with an incidence of 4.5 cases per million individuals aged less than twenty years [7] [8]. RMS has six histologic sub-types [8] [9], with major sub-types being alveolar and embryonal [2] [10] [11] [12]. Embryonal rhabdomyosarcoma (ERMS) represents about 80% of the cases [7], is more prevalent in children less than 10 years of age [13] [14], and has better prognosis and event-free survival of 81% [7] [13] [15]. It's associated with distinct genetic alterations and loss of heterozygosity at chromosome 11p15 locus [7], and possesses amplifications in *MYCN* and lesions in insulin receptors [16], mutations in *TP53* and *FGFR4* [15].

MYC proto-oncogene participates in many cellular processes including cell proliferation, differentiation and apoptosis [17]. It is the most frequent proto-oncogene that is amplified in 40% of numerous human cancers [18] [19]. Missiaglia *et al.* (2009) have reported a high amplification and overexpression of the oncogene *MYC* in the ERMS (RD) cells and other primary tumors [20]. Targeting and inhibition of *MYC* have also been shown to reduce the progression and tumorigenicity of RMS and myogenic differentiation [21], indicating *MYC* as a potential target for rhabdomyosarcoma treatment. Though *MYC* is under tight regulation in normal cells through various signaling pathways like WNT, Hedgehog and receptor tyrosine kinases, constitutive activation of these pathways results *MYC* activation in cancer cells [22] [23]. PI3K drug resistance was also associated with *MYC* amplification illustrating this gene as downstream of PI3K for tumorigenesis [22]. Inhibition of *MYC* expression, interrupting *Myc-Max* dimerization and final alteration of *Myc-Max* binding to DNA is crucial to

hinder target *MYC* genes [18].

The Notch signaling pathway involves regulation and determination of cell destiny and is dysregulated in human cancers [24]. This pathway can be targeted by inhibition of notch receptors using small molecule inhibitors [25]. The enzyme, gamma-secretase leads to the increase in the Notch intercellular domain and overactivation of the Notch pathway followed by transcriptional activation in the nucleus [26]. Belyea *et al.* (2011) showed using Notch1 RNAi or Y-secretase small molecule inhibitors reduce ERMS growth *in vitro* [25]. A study by Roma *et al.* (2011) have shown upregulation of the Notch pathway in fusion positive (FP) and fusion negative (FN) RMS with notable correlation between the invasiveness and motility of RMS cells up on Notch pathways activation [27]. The motility and invasiveness of the cells also reduced with gamma-secretase inhibition in *in vitro* models [27]. Furthermore, *NOTCH1* was shown to cause tumor propagation in embryonal RMS zebrafish models via upregulation of *SNAIL1* transcription factor, ultimately leading to dedifferentiation of the embryonal RMS cells [28]. The study also indicated the possible role of *NOTCH1*/*SNAIL1*/*MEF2C* pathway in the metastatic capacity of embryonal RMS [28]. Novel compounds that can inhibit this pathway will form promising therapeutic agents to treat metastatic cancers [29].

Fibroblast growth factor receptor (FGFR) belongs to a signaling pathway that is involved in diverse biological processes including growth, survival, differentiation, angiogenesis, tumor growth and development [30] [31]. Understanding the different roles of the fibroblast growth factor receptor family in cancer development will elucidate the importance of this pathway as potential target for cancer treatment and the development of new target therapies [32]. Mouron *et al.* (2021) reported that 25% of hormone receptor positive (HR+) breast cancer patients have amplification or overexpression of *FGFR1* [33]. Studies have reported the role of the Fibroblast growth factor receptor 1 (*FGFR1*) in the tumorigenesis of breast cancer and mammary development [34] [35]. Additionally, amplifications and overexpression of this factor (*FGFR1*) has been linked to poor prognosis of in breast cancer, functioning as a predictor of poor outcome [36] [37]. *FGFR1* has also been reported to be overexpressed in RMS [32]. A study by Missiaglia *et al.* (2009) has confirmed the amplification and gain of *FGFR1* and its overexpression in RMS cells with higher expressions in embryonal type than alveolar sub-type [20]. Goldstein *et al.* (2007) reported *FGFR1* overexpression in RMS cells with hypomethylations at the promoter region [38]. These data suggest *FGFR1* as a potential therapeutic target for RMS and search for therapeutic compounds that can target this gene and its pathway specifically [38]. Recent reports showed the correlation between resistance to chemotherapy and FGF/FGFR signaling and underscore drugs that target this signaling pathway as potential for cancer treatment [39].

The chemokine *CXCR7* is a seven transmembrane receptor that was re-named *ACKR3* as it is a member of the atypical chemokine receptor family (ACKR) [40]. Overexpression of *CXCR7* was shown to be important in the angiogenic

process in cancers, suggesting its role in tumor progression and its regulatory activity in the process of angiogenesis [40]. Sheng *et al.* (2010) showed the role of *CXCR7* in endothelial proliferation, migration and production of vascular endothelial growth factors that enhance tumor propagation through mediation of angiogenesis [41]. Embryonal RMS cells have been shown to highly express the *CXCR7* chemokine [42]. This chemokine was also reported to respond to stimulation by stromal derived factor (SDF)-1 also known as CXCL12, leading to ultimate activation of MAPK and AKT pathways. This activation was linked to the motility of RMS cells rather than proliferation and survival [43]. There is focus on small molecules as CXCR7 antagonists which show different affinities [40]. Simultaneous blocking of CXCR7 and CXCR4 also represents therapeutic approach as they involve both in cancer malignance [44].

Chemotherapy, surgery, radiation and their combination are the standard treatments for cancer. However, these are associated with limitations including lack of specificity, toxicity and development of drug resistance [45]. Therefore, the need to search for alternative therapeutic agents with less tendency for development of resistance and precisely target cancer cells [23]. Recently, there has been growing interest in antimicrobial peptides (AMPs) as a source of therapeutic agents with low development of resistance, higher efficacy and less toxicity to the cells [46] [47] [48]. Antimicrobial peptides (AMP) are short cationic peptides that are amphipathic in nature [49] [50], with diverse amino acids and secondary structures [51]. Recent studies suggest that antimicrobial peptides kill cancer cells by two mechanisms; membrane disruption leading to necrosis [52] [53], induction of apoptosis resulting from their binding to the mitochondrial membrane [54] [55]. Papo *et al.* (2004) showed that the D-K_6L_9 peptide (15-mer D,L-amino acid peptide) inhibits proliferation of prostate adenocarcinoma cells after intratumoral injection with no activity on non-malignant cells [56]. Systematic administration of this peptide also inhibited growth of primary and metastatic tumors [57]. Dermaseptin B2 [58], an α-helical polypeptide, is the most potent within the family of Dermaseptins, with activities in micromolar ranges [59] [60]. *In vitro* studies reported the antitumor and antiangiogenic activities of the antimicrobial peptide Dermaseptin B2 (Drs B2) [61]. Zoggel *et al.* (2012) demonstrated that Drs B2 inhibits colony formation of PC3 and MDA-MB231 tumor cells, the proliferation and capillary formation of endothelial HUVEC cells [62]. This later study reported necrotic effects of Drs B2 rather than apoptotic activities with no activation of caspase-3 and no changes in mitochondrial potential [62]. In the current study, we investigated the antiproliferative activity of Drs B2 against RMS (RD) cells and evaluated the activity of this peptide at the molecular level by assessing the expression of genes involved in the proliferation of cells (*MYC*), angiogenesis (*FGFR1*, *CXCR7*) and metastasis (*NOTCH1*, *CXCR7*).

2. Materials and Methods

2.1. Chemicals and Reagents

Dermaseptin B2 (Adenoregulin, lot no: 2020/10/14), Doxorubicin (cat no:

SD9280), and resazurin dye (Lot no: 415E035) were purchased from Solar Bio (Beijing, China). DMEM (Lot no: RNBH6554), FBS (Lo no: BCCB7351) and PBS (Batch no: 017K8212) were purchased from Sigma Aldrich. L-glutamine (Lot no: 2085472), Trypsin-EDTA (Lot no: 2091549), Penicillin-Streptomycin (Ref 1514-122) and Gentamycin (lot no: 2163664) were all purchased from Gibco (Gibco Biosciences). All the reagents were cell culture grade.

2.2. Specifications for Dermaseptin B2

Table 1 represents information on the antimicrobial peptide Dermaseptin B2 (Drs B2) used in this study.

2.2.1. Cell Culture

Cell lines RD (ATCC® CCL-136) and Vero (ATTC CCL-81) were purchased from the American Type Culture Collection (ATCC) and cultured as previously described [5] [63]. Briefly, cells were thawed in 37˚C water bath, disinfected with 70% ethanol, and carefully transferred into T-75 cell culture flasks and complete media containing DMEM, 10% FBS and penicillin/streptomycin added and incubated at 37˚C, 5% CO_2 and 95% humidity incubator. Cells were passaged every 3 - 4 days at 80% - 90% confluence level.

2.2.2. Determination of Cell Viability Using Resazurin Reduction Assay

The resazurin reduction assay was performed as previously described [64] [65]. At 65% - 75% Cells were trypsinased, counted with hemocytometer and plated into 96-well cell culture plates at seeding density of 1×10^4 cells/well and incubated in an incubator at 37˚C, 5% CO_2 and 95% humidity. The previous media was gently removed from the cells and 100 µL of increasing concentrations (3, 6, 9, 12 and 15 µM) of Drs B2 in complete media was added to each well in triplicates. Doxorubicin used as standard control drug was prepared the same way. Plates were then incubated for 24, 48, 72-hours to determine the anti-proliferative activity of Drs B2 compared to Doxorubicin. After 24, 48, 72-hours of incubation, 20 µL (0.15 mg/mL) of resazurin dye was added and incubated for 4-hours. The optical density of the cultures was then read with plate reader (Infinite M1000, Tecan), at an absorbance of 570 nm and 600 nm. The percentage cell viability was calculated from the net absorbance of 570 nm and 600 nm readings using the equation; percentage cell viability (% viability) = (Net absorbance of the treated cells/Net absorbance of the blank) × 100 [66]. The effect of the Drs B2 on the proliferation of the cells was then presented in graphs of % cell viability

Table 1. Basic information of dermaseptin B2 (Adenoregulin).

Names (CRO1001)	Dermaseptin B2 (Drs B2), Adenoregulin
UniProt Accession number	P31107 (DRS2_PHYBI)
Peptide Sequence	GLWSKIKEVGKEAAKAAAKAAGKAALGAVSEAV-NH2
Molecular weight	3180 Daltons
Organism	*Phyllomedusa bicolor* (Two-colored leaf frog)

against the log concentration (μM) of the treatment. The 50% inhibitory concentration (IC_{50}) of the treatments was then calculated using non-linear regression analysis in GraphPad Prism software (v8.4.3).

2.2.3. Cell Migration Assay

The 2-dimensional wound healing assay was performed as previously described [67] [68] [69]. RD cells were grown in T-75 cell culture flasks using DMEM supplemented with 10% FBS. Cells were trypsinased, counted and plated in 12-well plates with a cell density of 5×10^4 cells/well. Cell growth was monitored until the cells attained 95% - 100% confluence [68]. Plates were removed from the incubator and using sterile conditions in the biosafety cabinet, a 200 μL pipette tip was used to make a vertical wound on the cell monolayer. The cell debris together with the media was then removed and 2 ml of media containing either Drs B2 and Doxorubicin at their pre-determined IC_{50} was added to the wells. The blank (media with PBS solvent) was added to the untreated cells. Up on treatment, cells were observed under the inverted microscope, snapshot pictures taken and marked as time zero. At time points of 6, 24, 48 and 72-hours, cells were observed under the microscope and pictures were taken at each time point. Results were presented as the effects of the treatments on the cell recovery compared to the untreated control cells.

2.3. Gene Expression Analysis

2.3.1. RNA Extraction

Exponentially dividing RD cells with a confluence of 70% were treated with the corresponding calculated IC_{50} values of Drs B2 and Doxorubicin for time-points of 24, 48, 72-hours. Total RNA extraction was performed using DirectZol RNA Miniprep kit (Cat no: R2053, Zymo Research, USA) following manufacturer's instructions. Briefly, cells were lysed in TriZol lysis buffer, and an equal volume of ethanol was added (95% - 100%), and purified by adding to the Zymo SpinTM column. Cells were then washed and RNA was eluted in DNase free water. Purity and integrity of RNA was checked in nanodrop spectrophotometer (Thermofisher) and 1% agarose gel electrophoresis.

2.3.2. cDNA Synthesis

cDNA synthesis was performed using FIREScript RT cDNA Synthesis kit (Cat no: 06-15-00050, Solis BioDyne, Estonia) following manufacturer's instructions. A 20 μL reaction mix contained 10 μl of (500 ng/μl) of RNA, 1 μl Oligo (dT) primer (100 μM), 2 μl of 10x RT Reaction Buffer with DTT, 0.5 μl dNTP Mix (20 mM), 1 μl FIREScript RT, 0.5 μl RNase Inhibitor (40 U/μl) and 5 μl of nuclease-free water. Reverse transcription was performed using GeneAmp PCR system 9700 (Applied Biosystems, USA). The reaction was performed at 55˚C for 30 minutes and enzyme inactivation at 85˚C for 5 minutes.

2.3.3. Primer Designing and Optimization

The primers used in this study were designed using the National Center for Bio-

technology Information's (NCBI) Primer Blast tool https://www.ncbi.nlm.nih.gov/tools/primer-blast. Reference sequences of the genes were retrieved from GenBank in FASTA format and used to design the individual sequences for forward and reverse primers. A range of 70 - 300 bps, 40% - 60% GC content, and self-complementarity not exceeding 2 were targeted. To confirm the PCR products sizes, primer sequences were checked using the Sequence Manipulation Suite (SMS). Primers sequences and optimization conditions are presented in **Table 2**.

2.3.4. qRTPCR Analysis

The expression levels of the target genes were assessed using qRTPCR real time thermal cycler qTOWER^3 84 GmbH (Analytik Jena). The qRTPCR reaction was performed using Luna® Universal qRTPCR Master Mix (NEBM3003S, New England Biolabs) following manufacturer's instructions. A 20 µl reaction contained 10 µl of the Luna® master mix, 0.5 µl each of the forward and reverse primers (10 pmol/µl), 2 µl of cDNA and 7 µl of nuclease-free water. All reactions were carried out in triplicate. The qRTPCR was done using the program; initial denaturation: 95˚C for 60 seconds, 40 cycles of; denaturation: 95˚C for 15 seconds, annealing/extension: 62˚C for 30 seconds, melting at 60˚C - 95˚C. Data were analyzed using Microsoft excel software (version 2019). Expression levels of the target genes were determined by using the $2^{\wedge -ddCt}$ method [70], with reference to *β-Actin* housekeeping gene. **Figure 1** shows the qPCR products of the target genes.

2.3.5. Relative Gene Expression

The relative expression levels of the target genes were calculated using the $2^{\wedge -ddCt}$ method. The house-keeping gene *β-Actin* was used as reference gene to normalize the expression of the target genes. The relative expression was then presented as fold change relative to the controls.

Table 2. Primer sequences, annealing temperature and PCR product sizes of the target genes and their NCBI accession numbers.

Gene name		Sequence	Annealing Temp/˚C	PCR product size/bp	NCBI Ref Seq.	PCR Conditions
NOTCH1	F	5'GTGGGCTCCCGTGTTTTGTA3'	62	300	NM_017617.5	Initial denaturation: 95˚C for 3 min, 30 cycles of; Denaturation: 95˚C for 15 s, annealing: 62˚C for 45 s, Elongation: 72˚C for 3 min, Final Elongation: 72˚C for 10 min
	R	5'TCCCTCACTGGCATGACACA3'				
CXCR7	F	5'ATTTGATTGCCCGCCTCAGA3'	62	149	NM_020311.3	
	R	5'GACGCTTTTGTTGGGCATGT3'				
MYC	F	5'TGTTGAAATGGGTCTGGGGG3'	62	129	NM_002467.6	Initial denaturation: 95˚C for 5 min, 30 cycles of; Denaturation: 95˚C for 30 s, annealing: 62˚C for 1 min, Elongation: 72˚C for 3 min, Final Elongation: 72˚C for 10 min
	R	5'TCTCACCTTCTCACCTGCCT 3'				
FGFR1	F	5'ATTTCTGCCTTGGCCCTACC3'	62	175	NM_001354369.2	
	R	5'CTAGCGCAGTCTTTGGGGAA3'				
β-Actin	F	5'CGGCATCGTCACCAACTG3'	62	153	NM_001101.5	
	R	5'ACATGATCTGGGTCATCTTCTC3'				

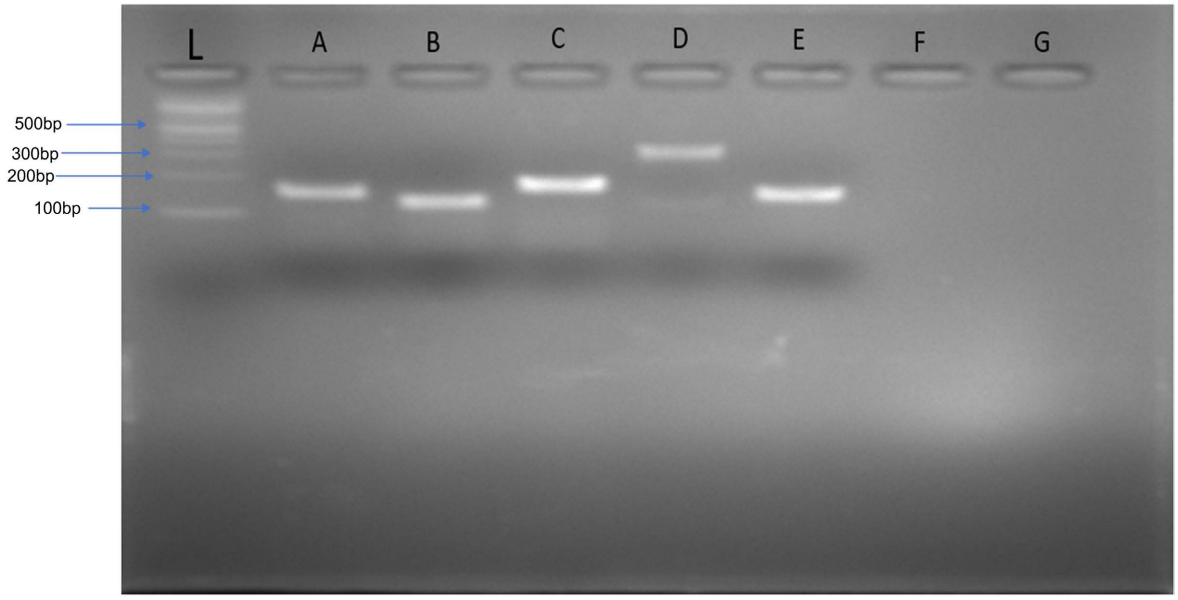

Figure 1. A 4% agarose gel of the optimized primers of the target genes: **L**: 100 bp ladder, **A**: *β-ACTIN* (153 bp), **B**: *MYC* (129 bp), **C**: *FGFR1* (175 bp), **D**: *NOTCH1* (300 bp), **E**: *CXCR7* (149 bp).

2.4. Data Analysis

All experiments were performed in triplicates of at least three replicates. Data were presented in Microsoft Excel software (version 2019) and expressed as mean ± SE. Analysis of variance was carried out using Graph-pad Prism software (v8.4.3) and used to compare the difference between the groups. *P. value* < 0.05 was considered as statistically significant.

3. Results

3.1. Effect of Dermaseptin B2 on the Proliferation of RD Cells

The antiproliferative activity of Drs B2 and Doxorubicin was assessed against rhabdomyosarcoma (RD) cell line. Treatments were performed in time-points of 24, 48, and 72-hours. Doxorubicin was used as positive a control. Results indicate that treatments beyond 9 µM lead to complete inhibition of cell proliferation. The inhibitory concentrations (IC50) of Drs B2 and Doxorubicin for the time-points of 24-hours (**Figure 2**), 48-hours (**Figure 3**) and 72-hours (**Figure 4**) are presented in **Table 3**. The data reveals that, the activity of both Drs B2 (5.993 µM) and Doxorubicin (1.886 µM) increases with longer incubation (72-hours) of the cells with the treatments. Though Doxorubicin showed greater inhibition of the cell growth, when these results were compared using analysis of variance (ANOVA), they did not show a significant difference between Drs B2 and Doxorubicin in all of the treatment time-points.

3.2. Effect of Drs B2 on Cell Migration

The effect of Drs B2 and Doxorubicin on cell migration is depicted in **Figure 5**. The normal morphology of the RD cells before each treatment are shown (letters

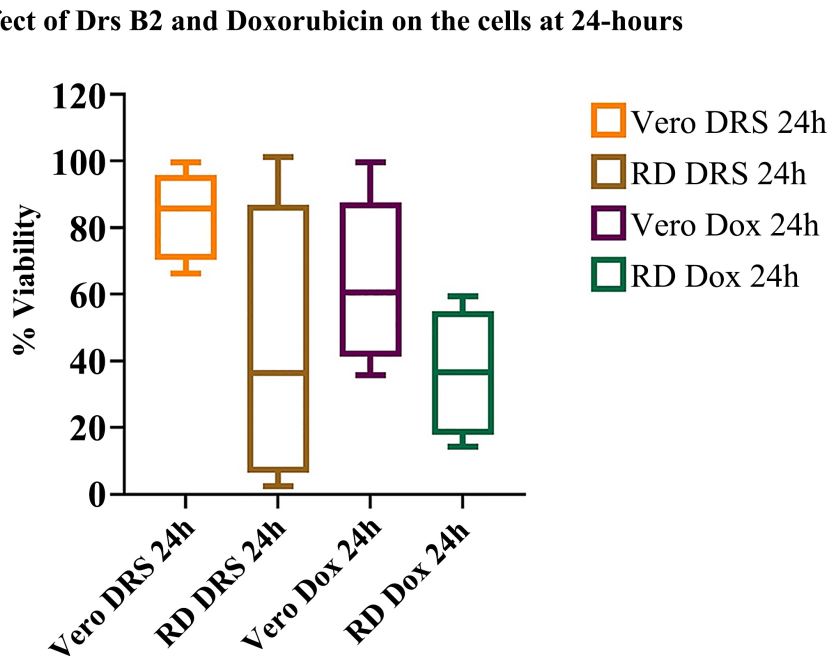

Figure 2. Effects of Drs B2 and doxorubicin on the cells for 24-hour treatment. Cells were cultured in DMEM and plated in 96-well plates after which treatment was performed. Cell viability was determined by staining cells with Resazurin cell viability dye and incubated for 24-hours and plates were read on plate reader Infinite M1000 (Tecan) machine. Each experiment was performed at least in three replicates. Results were presented as mean ± SE.

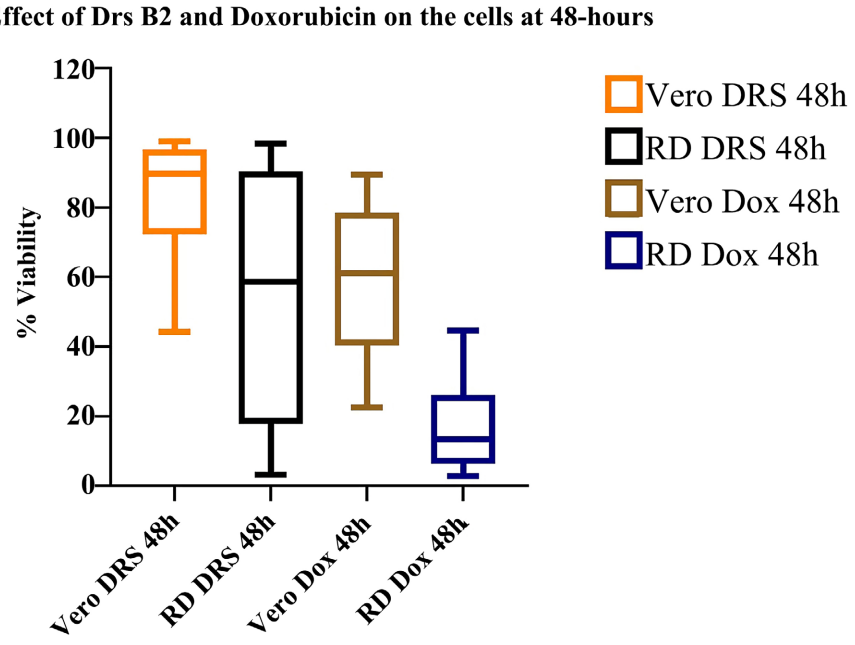

Figure 3. Effects of Drs B2 and doxorubicin on the cells for 48-hour treatment. Cells were cultured in DMEM and plated in 96-well plates after which treatment was performed. Cell viability was determined by staining cells with Resazurin cell viability dye and incubated for 4-hours and plates were read on plate reader Infinite M1000 (Tecan). Each experiment was performed at least in three replicates. Results were presented as mean ± SE.

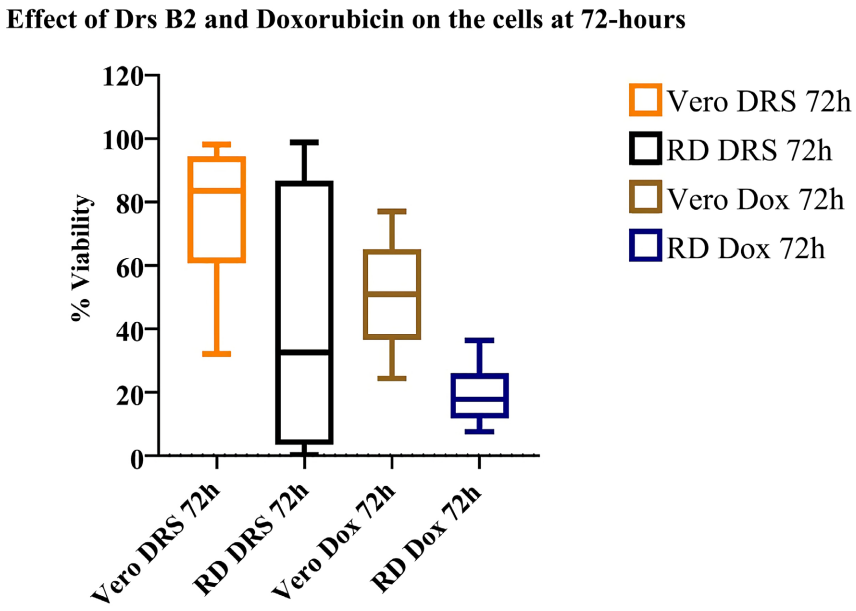

Figure 4. Effects of Drs B2 and doxorubicin on the cells for 72-hour treatment. Cells were cultured in DMEM and plated in 96-well plates after which treatment was performed. Cell viability was determined by staining cells with Resazurin cell viability dye and incubated for 4-hours and plates were read on plate reader Infinite M1000 (Tecan). Each experiment was performed at least in three replicates. Results were presented as mean ± SE.

Table 3. IC_{50} values for Drs B2 and doxorubicin treatments on RD cells during the time interval.

CELL TYPE	Drs B2 IC_{50} (μM)			Doxorubicin IC_{50} (μM)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
RD cells (IC_{50})	7.679	7.235	5.993	4.879	2.701	1.886
Vero cells (CC_{50})	20.53	13.87	11.64	11.17	8.138	6.890
Selectivity Index (SI) = CC_{50}/IC_{50}	2.673	1.917	1.942	2.289	3.079	3.653

CC_{50}: cytotoxic concentration, IC_{50}: Inhibitory concentration, Drs B2: dermaseptin B2, RD: Rhabdomyosarcoma cells.

An, Bn, Cn). A0, A6, A24, A48 and A72 represent the wound closure of the untreated cells (blank control). This result shows that blank control did not have effect on cell recovery during the treatments intervals. B0, B6, B24, B48 and B72 show the effect of Drs B2 on the wound closure of the RD cells. This result indicates that Drs B2 inhibited the cell migration and affected the ability of the cells to recover, with longer incubation (48, 72-hours) showing stronger effects. Conversely, C0, C6, C24, C48 and C72 represent the effect of Doxorubicin on the wound closure of the cells. Doxorubicin also inhibited cell migration and affected the recovery of the cells toward wound closure. Altogether, these results show the effect of Drs B2 and Doxorubicin on cell migration and motility, which is important in the spread of cancer cells and their metastatic process.

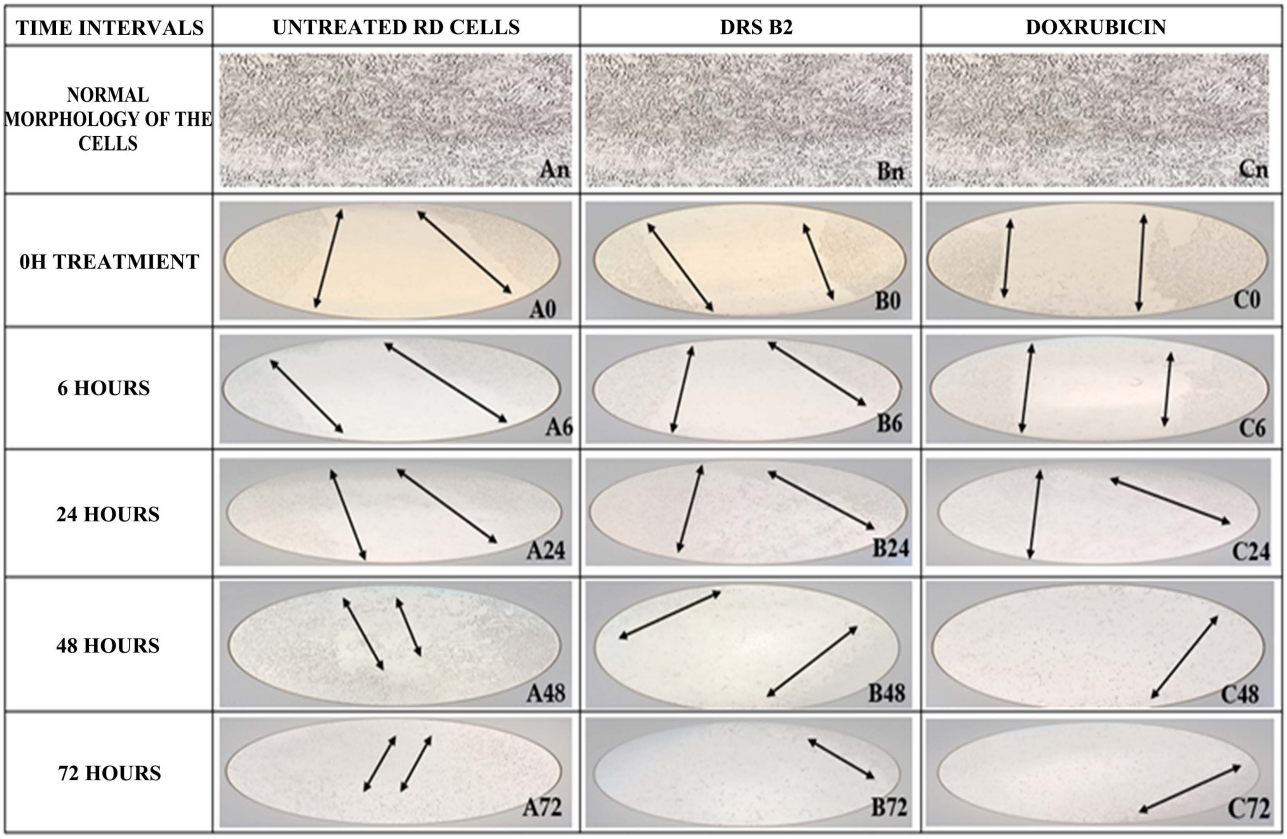

Figure 5. Wound healing assay. Effect of Drs B2 and doxorubicin on the migration of the cells. Time intervals presented as 0, 6, 24, 48, and 72-hours of incubation. Arrows indicate the space of the wound during the time-points of the treatments. Images were captured in 10× magnification.

3.3. Gene Expression Analysis

The effect of Drs B2 on the expression profiles of *MYC*, *NOTCH1*, *FGFR1*, and *CXCR7* was investigated. Relative gene expression was carried out by comparing the expression profiles of the target genes between the treated and untreated RD cells and the results were normalized to the reference gene *β-Actin*. The Livak [70] method was used to determine the fold change expression of the target genes between the treated and untreated cells. Both Drs B2 and Doxorubicin significantly downregulated the expression of all the target genes. Same letters on top of bars indicate no significant differences, while different letters indicate significant difference using Tukey's multiple comparison tests (≤0.05) carried out in GraphPad Prism software (v8.4.3).

Effect of Drs B2 and Doxorubicin on the expression of *MYC* in RD cells at 24, 48 and 72-hours is shown in **Figure 6**. At 72-hour treatment (*P. value* = 0.01), *MYC* was significantly downregulated in the RD cells treated with Drs B2 (fold change = 2.4144) and Doxorubicin (fold change: 3.5067). Fold change expressions for all the genes are shown in **Table 4**. There were no significant differences in the 24-hour and 48-hour time-points between the treated and untreated cells (*P. value* = 0.4). Conversely, no significant differences were found between Drs B2 and Doxorubicin in all of the treatment time-points (*P. value* = 0.53).

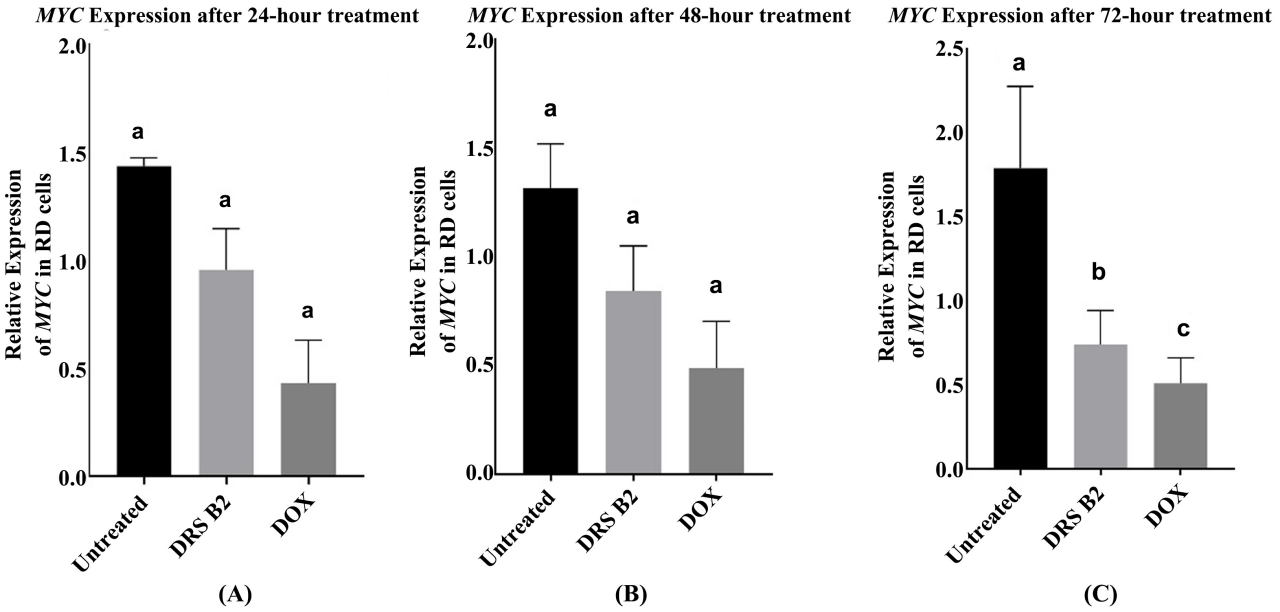

Figure 6. *MYC* expression in RD cells after 24, 48 and 72-hour treatment with Drs B2 and doxorubicin. (A) *MYC* expression after 24-hour treatment. (B) *MYC* expression after 48-hour treatment. (C) *MYC* expression after 72-hour treatment. Each treatment was performed in three replicates.

Table 4. Relative fold changes of the target genes during the treatment intervals.

Time interval	Relative fold changes of the target genes after treatment of cells with Drs B2 and Doxorubicin							
	Drs B2				Doxorubicin			
	MYC	*NOTCH1*	*CXCR7*	*FGFR1*	*MYC*	*NOTCH1*	*CXCR7*	*FGFR1*
24 hours	1.5013	2.4667	2.8818	2.3515	2.3483	3.0189	3.9366	2.5943
48 hours	1.5185	4.6274	4.4430	2.0809	2.7945	4.7481	5.8937	2.3105
72 hours	2.4144	4.3352	3.9924	2.2543	3.5067	5.7878	5.5799	2.2402

Figure 7 shows the effect of Drs B2 and Doxorubicin on the expression of *FGFR1*. At *P. value*: 0.01, both Drs B2 and Doxorubicin significantly downregulated *FGFR1* in the treated cells at 24, and 48-hours treatments. This downregulation was also significant at the 72-hour (*P. value*: 0.003) treatment for both Drs B2 and Doxorubicin. Fold change expressions are presented in Table 4. There was slight reduction in the fold change expression in the 72-hour treatments. No significant difference was observed between Drs B2 and Doxorubicin between all of the treatment time-points (*P. value*: 0.9).

The effect of Drs B2 and Doxorubicin on the expression of *NOTCH1* gene is shown in Figure 8. At *P. value* 0.0001, both Drs B2 and Doxorubicin downregulated the *NOTCH1* gene in all the treatment intervals of 24, 48 and 72-hour in the treated cells compared to the untreated cells. Fold change expressions are presented in Table 4. No significant difference was observed between Drs B2 and Doxorubicin in all of the treatment time-points (*P. value* = 0.58).

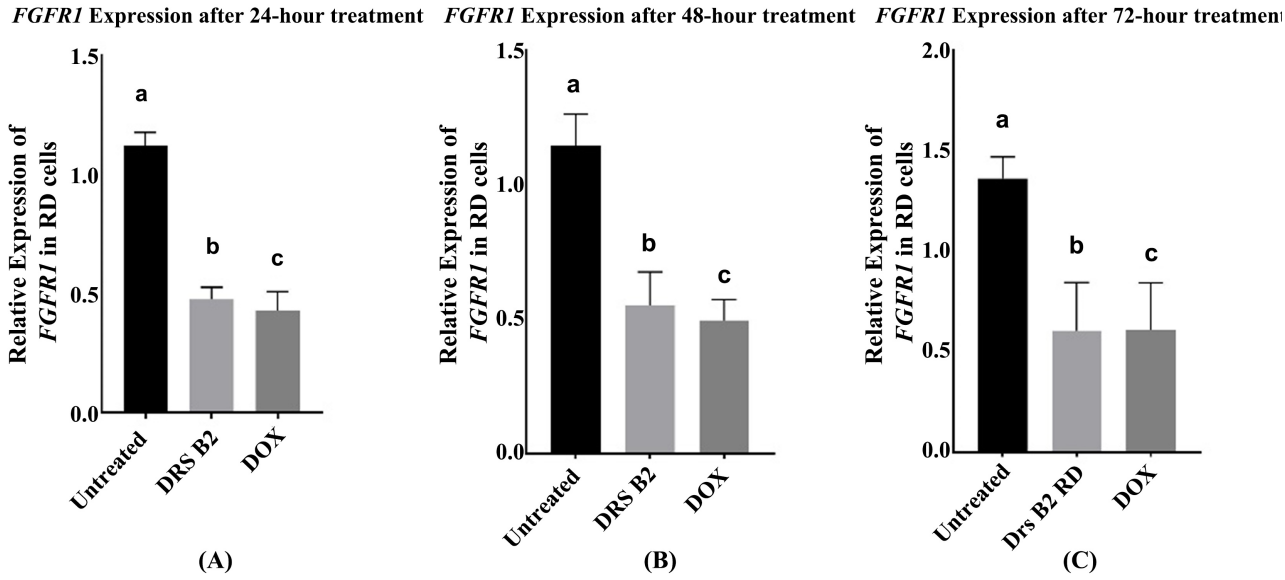

Figure 7. *FGFR1* expression in RD cells after 24, 48 and 72-hour treatment with Drs B2 and doxorubicin. (A) *FGFR1* expression after 24-hour treatment. (B) *FGFR1* expression after 48-hour treatment. (C) *FGFR1* expression after 72-hour treatment. Each treatment was performed in three replicates.

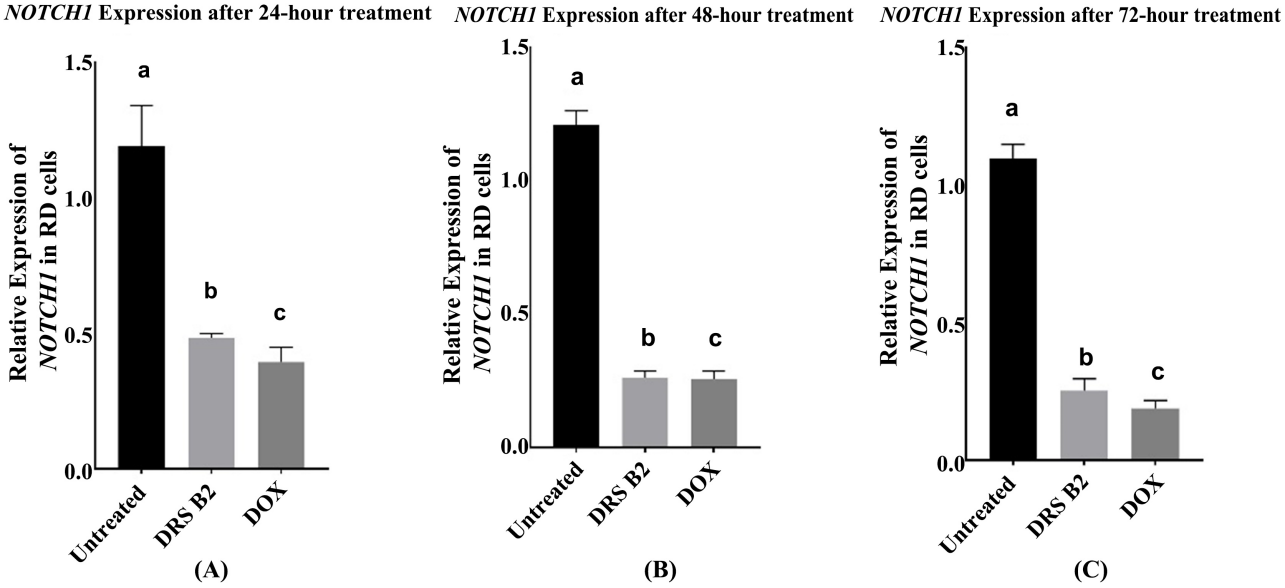

Figure 8. *NOTCH1* expression in RD cells after 24, 48 and 72-hour treatment with Drs B2 and doxorubicin. (A) *NOTCH1* expression after 24-hour treatment. (B) *NOTCH1* expression after 48-hour treatment. (C) *NOTCH1* expression after 72-hours treatment. Each treatment was performed in three replicates.

The effect of Drs B2 and Doxorubicin on the expression of CXCR7 gene is shown in Figure 9. At P. value 0.0001, both Drs B2 and Doxorubicin downregulated the CXCR7 gene in all the treatment time-points of 24, 48 and 72-hour in the treated cells in comparison to the untreated cells. Fold change expressions are presented in Table 4. There was no significant difference between Drs B2 and Doxorubicin in of the treatment time-points.

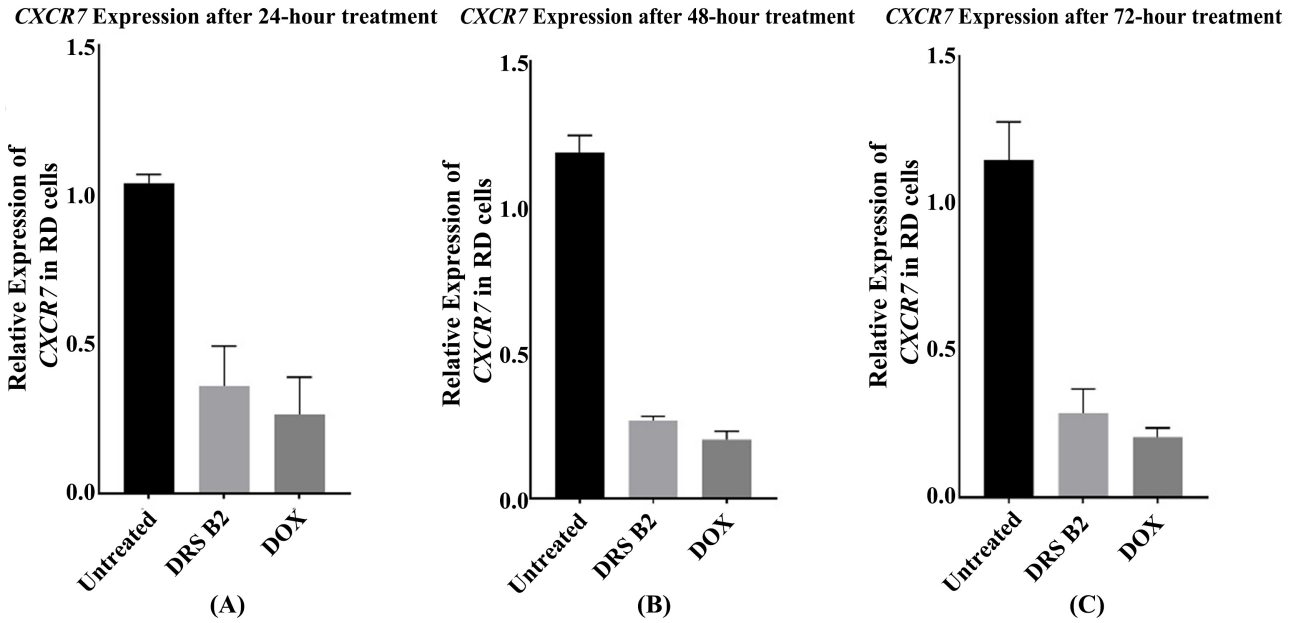

Figure 9. *CXCR7* expression in RD cells after 24, 48 and 72-hour treatment with Drs B2 and doxorubicin. (A) *CXCR7* expression after 24-hour treatment. (B) *CXCR7* expression after 48-hour treatment. (C) *CXCR7* expression after 72-hour treatment. Each experiment was performed in three replicates.

4. Discussion

The current study sought to investigate the antiproliferative activity of the antimicrobial peptide Drs B2 against rhabdomyosarcoma (RD) cells and its effect on expression of genes involved in the proliferation of cells (*MYC*), angiogenesis (*FGFR1*, *CXCR7*) and metastasis (*NOTCH1*, *CXCR7*), while using doxorubicin as a control anti-cancer drug. Results indicated that Drs B2 has strong antiproliferative activity against rhabdomyosarcoma (RD) cells in time-dependent manner and the inhibitory action of Drs B2 was deducted as time and concentration dependent. These findings were in line with previous studies which reported the inhibition of cell growth by Drs B2 against various cancer cells with micromolar concentrations [62]. In comparison, a Dermaseptin-L1, member of the Dermaseptin family, was reported to have selective inhibitory activity (GI_{50}) of 45μM [71], suggesting the higher activity of Drs B2 compared to other family members of Dermaseptins. These results notwithstanding, in comparison with Doxorubicin, Drs B2 showed less specificity given the lower selectivity index towards Vero Cells, however, the difference is not significant, thus Drs B2 can be a potential alternative for treatment.

One of the main complications associated with cancer treatment is due to metastasis to distant organs leading to poor prognosis and survival [72]. Study of cell migration is an important aspect of cell's motility and their ability to metastasize. Drs B2 and Doxorubicin strongly inhibited the migration of the cells and prolonged exposure (48 and 72-hours) resulted in the failure of the cells to re-establish the wound/scratch. Inhibition of cell's communication and recovery through contact inhibition is an indication of their inability to move and hence

interference of their metastatic potential [72]. This result shows that Drs B2 affects factors or genes that involve in cell migration and invasiveness. This result is also in further support of the downregulation of *FGFR1* gene that involve metastatic process by Drs B2.

MYC plays many critical functions including proliferation, growth, and apoptotic process [21]. This gene has been shown to be dysregulated in different human cancers and its inactivation leads to tumor regression and can be targeted for cancer treatment [73]. The downregulation of *MYC* gene in RD by Drs B2 is an indication that it affected processes involving cell proliferation and growth. This result can be further supported by the cytotoxicity results of the treatments which showed greater inhibitory activity with longer incubation of the cells with the treatments. One of the main mechanisms of action of cancer therapeutic drugs is induction of apoptosis [74], Drs B2 could also play part the process, although a previous study reported that Drs B2 had more necrotic than apoptotic activity [62].

Ignatius *et al.* (2017) have shown the role of *NOTCH1* in embryonal rhabdomyosarcoma differentiation and its effect on overall tumorigenesis [28]. A previous study reported that Y-secretase inhibitors reduced the mobility of RMS cells but had no effect on cell cycle or apoptotic processes [27]. This indicates Notch pathway inhibition reduces the invasiveness of the cells, therefore, novel compounds that can inhibit this pathway could be of critical importance in RMS treatment and might have anti-metastatic potential. In this study, Drs B2 (fc: 4.6275) and Doxorubicin (fc: 5.7878), had significant effect on *NOTCH1* expression in the treated cells. Though fold change expression of *NOTCH1* slightly reduced for Drs B2 in the 72-hour (fc: 4.3352) interval, this gene was significantly downregulated all the treatment intervals (*P. value* 0.0001). These results can be further supported by the activity Drs B2 and Doxorubicin on cell migration which indicates their possible effect on factors or genes that involve in cell motility and invasiveness. The higher efficacy of Doxorubicin could be due to the fact that Doxorubicin is used in the management of later stages of RMS and metastatic solid tumors [8].

Fibroblast growth factor receptors (FGFRs) play important roles involving survival, motility, homeostasis and carcinogenesis processes [75]. Studies have confirmed the amplification and overexpression of *FGFR1* in RMS [20] [32] [38]. Therefore, novel therapeutic agents that can target and inhibit processes including neovascularization of the cells would be crucial in the treatment of RMS. Exposure of RD cells to both Drs B2 and Doxorubicin resulted in significant downregulation of *FGFR1* in all of the treatment intervals, though for both Drs B2 (fc: 2.3515, *p. value*; 0.01) and Doxorubicin (fc: 2.5943, *p. value*; 0.007), were more potent in the 24-hour intervals. Our results are in support of the previously reported angiostatic effects of Drs B2 [61]. As *FGFR1* involves many processes including neovascularization and angiogenesis, its downregulation in the cells is an indication of the novel anti-angiogenic activity of Drs B2 and inhi-

bition of the neovascularization in the RD cells.

CXCR7 was shown to be highly expressed RMS cells and was linked to increase in the adhesiveness of these cells [43]. It was also reported that CXCR7 has key roles in controlling angiogenic process [40]. The role CXCR7 in controlling the metastatic propagation, adhesion, and invasion of RMS cells was also reported [41] [43], indicating this gene axis as target for RMS treatment. The relative downregulation of CXCR7 in the treated cells compared to the untreated cells is further indication of the effect treatment of Drs B2 on neovascularization and the anti-angiogenic potential of Drs B2. Chemokines and their receptors regulate pro-metastatic propagation of RMS cells increasing motility, chemotaxis and expression of MMP as well as cell adhesion [76] [77] [78], but not directly involve in RMS cell growth, blocking of the newly identified SDF-1 binding receptor for CXCR7 [43], by Drs B2 might be target in these cells.

5. Conclusion

Drs B2 shows strong antiproliferative activity against RMS cells. Cell migration assay results showed that Drs B2 exhibits strong inhibitory activity on cell migration which could be due to its effect on factors that involve in cell migration and motility. Gene expression results showed that Drs B2 downregulates genes that involve in proliferation, angiogenesis and metastatic propagation of the cells. Further studies are needed to give a better understanding of the detailed mechanisms of action as well as the effects of the Dermaseptin B2 peptide *in vivo.*

Author Contributions

Conceptualization, Ahmed A. Abdille, Josephine Kimani, Fred Wamunyokoli and Esther. N Maina. Data curation, Ahmed A. Abdille; Formal analysis, Ahmed A. Abdille; Funding acquisition, Ahmed A. Abdille, Josephine Kimani, Fred Wamunyokoli and Esther. N Maina; Investigation, Ahmed A. Abdille; Methodology, Ahmed A. Abdille, Josephine Kimani, Fred Wamunyokoli and Esther. N Maina; Project administration, Fred Wamunyokoli; Resources, Josephine Kimani, Fred Wamunyokoli, Wallace Bulimo and Esther. N Maina; Supervision, Josephine Kimani, Fred Wamunyokoli and Esther. N Maina; Validation, Ahmed A. Abdille, Josephine Kimani, Fred Wamunyokoli, Wallace Bulimo, Yahaya Gavamukulya and Esther. N Maina; Writing—original draft, Ahmed A. Abdille; Writing—review & editing, Josephine Kimani, Fred Wamunyokoli, Wallace Bulimo, Yahaya Gavamukulya and Esther. N Maina. All authors read and approved the final version of the manuscript.

Funding

This study was financially supported by the African Union under the Pan African University Institute for Basic Sciences Technology and Innovation (PAUSTI), Grant number (MB400-0007/2019) and the AFRICA-*ai*-JAPAN Project (phase 2, 2021-2022).

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

All the raw data for this study can be obtained from the corresponding author up on reasonable request.

Acknowledgements

Authors extend thanks to the Pan African University for supporting this study. Many thanks are also extended to Mark Ndubi, Samwel Symekher, Silvanos Mukunzi, Meshack Wedagu, Janet Mananja, and the entire team at Kenya Medical Research Institute (KEMRI) Centre for Virus Research (CVR) at the Department of Emerging Infectious Diseases, Influenza clean cell culture laboratory for their generous support.

Conflicts of Interest

Authors declare no conflict of interest.

References

[1] Kommoss, F.K.F., Stichel, D., Mora, J., Esteller, M., *et al.* (2021) Clinicopathologic and Molecular Analysis of Embryonal Rhabdomyosarcoma of the Genitourinary Tract: Evidence for a Distinct DICER1-Associated Subgroup. *Modern Pathology*, **34**, 1558-1569. https://doi.org/10.1038/s41379-021-00804-y

[2] Kather, J.N., Hörner, C., Weis, C.A., *et al.* (2019) CD163+ Immune Cell Infiltrates and Presence of CD54+ Microvessels Are Prognostic Markers for Patients with Embryonal Rhabdomyosarcoma. *Scientific Reports*, **9**, Article No. 9211. https://doi.org/10.1038/s41598-019-45551-y

[3] Liu, Z., Zhang, X., Lei, H., *et al.* (2020) CASZ1 Induces Skeletal Muscle and Rhabdomyosarcoma Differentiation through a Feed-Forward Loop with MYOD and MYOG. *Nature Communications*, **11**, Article No. 911. https://doi.org/10.1038/s41467-020-14684-4

[4] Pomella, S., Sreenivas, P., Gryder, B.E., *et al.* (2021) Interaction between SNAI2 and MYOD Enhances Oncogenesis and Suppresses Differentiation in Fusion Negative Rhabdomyosarcoma. *Nature Communications*, **12**, Article No. 192. https://doi.org/10.1038/s41467-020-20386-8

[5] Walter, D., Satheesha, S., Albrecht, P., *et al.* (2011) CD133 Positive Embryonal Rhabdomyosarcoma Stem-Like Cell Population Is Enriched in Rhabdospheres. *PLoS ONE*, **6**, e19506. https://doi.org/10.1371/journal.pone.0019506

[6] Hoang, N.T., Acevedo, L.A., Mann, M.J. and Tolani, B. (2018) A Review of Soft-Tissue Sarcomas: Translation of Biological Advances into Treatment Measures. *Cancer Management and Research*, **10**, 1089-1114. https://doi.org/10.2147/CMAR.S159641

[7] Chen, C., Dorado Garcia, H., Scheer, M. and Henssen, A.G. (2019) Current and Future Treatment Strategies for Rhabdomyosarcoma. *Frontiers in Oncology*, **9**, 1458. https://doi.org/10.3389/fonc.2019.01458

[8] Bisogno, G., Jenney, M., Bergeron, C., *et al.* (2018) Addition of Dose-Intensified Doxorubicin to Standard Chemotherapy for Rhabdomyosarcoma (EpSSG RMS 2005): A Multicentre, Open-Label, Randomised Controlled, Phase 3 Trial. *The Lancet Oncology*, **19**, 1061-1071. https://doi.org/10.1016/S1470-2045(18)30337-1

[9] Amer, K.M., Thomson, J.E., Congiusta, D., *et al.* (2019) Epidemiology, Incidence, and Survival of Rhabdomyosarcoma Subtypes: SEER and ICES Database Analysis. *Journal of Orthopaedic Research*, **37**, 2226-2230. https://doi.org/10.1002/jor.24387

[10] Rossi, F., Legnini, I., Megiorni, F., *et al.* (2019) Circ-ZNF609 Regulates G1-S Progression in Rhabdomyosarcoma. *Oncogene*, **38**, 3843-3854. https://doi.org/10.1038/s41388-019-0699-4

[11] Lychou, S.E., Gustafsson, G.G. and Ljungman, G.E. (2016) Higher Rates of Metastatic Disease May Explain the Declining Trend in Swedish Paediatric Rhabdomyosarcoma Survival Rates. *Acta Paediatrica*, **105**, 74-81. https://doi.org/10.1111/apa.13172

[12] Li, Y., Bakke, J., Finkelstein, D., Zeng, H., Wu, J. and Chen, T. (2018) HNRNPH1 Is Required for Rhabdomyosarcoma Cell Growth and Survival. *Oncogenesis*, **7**, Article No. 9. https://doi.org/10.1038/s41389-017-0024-4

[13] Marshall, A.D. and Grosveld, G.C. (2012) Alveolar Rhabdomyosarcoma—The Molecular Drivers of PAX3/7-FOXO1-Induced Tumorigenesis. *Skeletal Muscle*, **2**, Article No. 25. https://doi.org/10.1186/2044-5040-2-25

[14] Hinson, A.R.P., Jones, R., Lisa, L.E., Belyea, B.C., Barr, F.G. and Linardic, C.M. (2013) Human Rhabdomyosarcoma Cell Lines for Rhabdomyosarcoma Research: Utility and Pitfalls. *Frontiers in Oncology*, **3**, 183. https://doi.org/10.3389/fonc.2013.00183

[15] Shern, J.F., Chen, L., Chmielecki, J., *et al.* (2014) Comprehensive Genomic Analysis of Rhabdomyosarcoma Reveals a Landscape of Alterations Affecting a Common Genetic Axis in Fusion-Positive and Fusion-Negative Tumors. *Cancer Discovery*, **4**, 216-231. https://doi.org/10.1158/2159-8290.CD-13-0639

[16] Comiskey, D.F., Jacob, A.G., Sanford, B.L., *et al.* (2018) A Novel Mouse Model of Rhabdomyosarcoma Underscores the Dichotomy of MDM2-ALT1 Function *in Vivo*. *Oncogene*, **37**, 95-106. https://doi.org/10.1038/onc.2017.282

[17] Patel, J.H., Loboda, A.P., Showe, M.K., Showe, L.C. and McMahon, S.B. (2004) Analysis of Genomic Targets Reveals Complex Functions of MYC. *Nature Reviews Cancer*, **4**, 562-568. https://doi.org/10.1038/nrc1393

[18] Dang, C.V. (2012) MYC on the Path to Cancer. *Cell*, **149**, 22-35. https://doi.org/10.1016/j.cell.2012.03.003

[19] Feng, Y.C., Liu, X.Y., Teng, L., *et al.* (2020) c-Myc Inactivation of p53 through the Pan-Cancer lncRNA MILIP Drives Cancer Pathogenesis. *Nature Communications*, **11**, Article No. 4980. https://doi.org/10.1038/s41467-020-18735-8

[20] Missiaglia, E., Selfe, J., Hamdi, M., *et al.* (2009) Genomic Imbalances in Rhabdomyosarcoma Cell Lines Affect Expression of Genes Frequently Altered in Primary Tumors: An Approach to Identify Candidate Genes Involved in Tumor Development. *Genes, Chromosomes & Cancer*, **48**, 455-467. https://doi.org/10.1002/gcc.20655

[21] Marampon, F., Ciccarelli, C. and Zani, B.M. (2006) Down-Regulation of c-Myc

Following MEK/ERK Inhibition Halts the Expression of Malignant Phenotype in Rhabdomyosarcoma and in Non Muscle-Derived Human Tumors. *Molecular Cancer*, **5**, Article No. 31. https://doi.org/10.1186/1476-4598-5-31

[22] Ilic, N., Utermark, T., Widlund, H.R. and Roberts, T.M. (2011) PI3K-Targeted Therapy Can Be Evaded by Gene Amplification along the MYC-Eukaryotic Translation Initiation Factor 4E (eIF4E) Axis. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, E699-E708. https://doi.org/10.1073/pnas.1108237108

[23] Muellner, M.K., Uras, I.Z., Gapp, B.V., *et al.* (2011) A Chemical-Genetic Screen Reveals a Mechanism of Resistance to PI3K Inhibitors in Cancer. *Nature Chemical Biology*, **7**, 787-793. https://doi.org/10.1038/nchembio.695

[24] Huang, Z., Lin, S., Long, C., *et al.* (2018) Notch Signaling Pathway Mediates Doxorubicin-Driven Apoptosis in Cancers. *Cancer Management and Research*, **10**, 1439-1448. https://doi.org/10.2147/CMAR.S160315

[25] Belyea, B.C., Naini, S., Bentley, R.C. and Linardic, C.M. (2011) Inhibition of the Notch-Hey1 Axis Blocks Embryonal Rhabdomyosarcoma Tumorigenesis. *Clinical Cancer Research*, **17**, 7324-7336. https://doi.org/10.1158/1078-0432.CCR-11-1004

[26] Kopan, R. (2002) Notch: A Membrane-Bound Transcription Factor. *Journal of Cell Science*, **115**, 1095-1097. https://doi.org/10.1242/jcs.115.6.1095

[27] Roma, J., Masià, A., Reventós, J., De Toledo, J.S. and Gallego, S. (2010) Notch Pathway Inhibition Significantly Reduces Rhabdomyosarcoma Invasiveness and Mobility *in Vitro*. *Clinical Cancer Research*, **17**, 505-513. https://doi.org/10.1158/1078-0432.CCR-10-0166

[28] Ignatius, M.S., Hayes, M.N., Lobbardi, R., Chen, E.Y., McCarthy, K.M., *et al.* (2017) The NOTCH1/SNAIL1/MEF2C Pathway Regulates Growth and Self-Renewal in Embryonal Rhabdomyosarcoma. *Cell Reports*, **19**, 2304-2318. https://doi.org/10.1016/j.celrep.2017.05.061

[29] Roma, J., Almazán-Moga, A., Sánchez de Toledo, J. and Gallego, S. (2012) *Notch*, *Wnt*, and *Hedgehog* Pathways in Rhabdomyosarcoma: From Single Pathways to an Integrated Network. *Sarcoma*, **2012**, Article ID: 695603. https://doi.org/10.1155/2012/695603

[30] Korc, M. and Friesel, R. (2009) The Role of Fibroblast Growth Factors in Tumor Growth. *Current Cancer Drug Targets*, **9**, 639-651. https://doi.org/10.2174/156800909789057006

[31] Zhou, W.Y., Zheng, H., Du, X.L. and Yang, J.L. (2016) Characterization of FGFR Signaling Pathway as Therapeutic Targets for Sarcoma Patients. *Cancer Biology & Medicine*, **13**, 260-268. https://doi.org/10.20892/j.issn.2095-3941.2015.0102

[32] Wesche, J., Haglund, K. and Haugsten, E.M. (2011) Fibroblast Growth Factors and Their Receptors in Cancer. *Biochemical Journal*, **437**, 199-213. https://doi.org/10.1042/BJ20101603

[33] Mouron, S., Manso, L., Caleiras, E., *et al.* (2021) FGFR1 Amplification or Overexpression and Hormonal Resistance in Luminal Breast Cancer: Rationale for a Triple Blockade of ER, CDK4/6, and FGFR1. *Breast Cancer Research*, **23**, Article No. 21. https://doi.org/10.1186/s13058-021-01398-8

[34] Suyama, K., Shapiro, I., Guttman, M. and Hazan, R.B. (2002) A Signaling Pathway Leading to Metastasis Is Controlled by N-Cadherin and the FGF Receptor. *Cancer Cell*, **2**, 301-314. https://doi.org/10.1016/S1535-6108(02)00150-2

[35] Xian, W., Schwertfeger, K.L., Vargo-Gogola, T. and Rosen, J.M. (2005) Pleiotropic

Effects of FGFR1 on Cell Proliferation, Survival, and Migration in a 3D Mammary Epithelial Cell Model. *Journal of Cell Biology*, **171**, 663-673. https://doi.org/10.1083/jcb.200505098

[36] Massabeau, C., Sigal-Zafrani, B., Belin, L., *et al.* (2012) The Fibroblast Growth Factor Receptor 1 (FGFR1), a Marker of Response to Chemoradiotherapy in Breast Cancer? *Breast Cancer Research and Treatment*, **134**, 259-266. https://doi.org/10.1007/s10549-012-2027-3

[37] Chen, L., Qi, H., Zhang, L., *et al.* (2018) Effects of FGFR Gene Polymorphisms on Response and Toxicity of Cyclophosphamide-Epirubicin-Docetaxel-Based Chemotherapy in Breast Cancer Patients. *BMC Cancer*, **18**, Article No. 1038. https://doi.org/10.1186/s12885-018-4951-z

[38] Goldstein, M., Meller, I. and Orr-Urtreger, A. (2007) FGFR1 Over-Expression in Primary Rhabdomyosarcoma Tumors Is Associated with Hypomethylation of a 5' CpG Island and Abnormal Expression of the AKT1, NOG, and BMP4 Genes. *Genes, Chromosomes & Cancer*, **46**, 1028-1038. https://doi.org/10.1002/gcc.20489

[39] Zhou, Y., Wu, C., Lu, G., Hu, Z., Chen, Q. and Du, X. (2020) FGF/FGFR Signaling Pathway Involved Resistance in Various Cancer Types. *Journal of Cancer*, **11**, 2000-2007. https://doi.org/10.7150/jca.40531

[40] Wang, C., Chen, W. and Shen, J. (2018) CXCR7 Targeting and Its Major Disease Relevance. *Frontiers in Pharmacology*, **9**, 641. https://doi.org/10.3389/fphar.2018.00641

[41] Zheng, K., Li, H.Y., Su, X.L., *et al.* (2010) Chemokine Receptor CXCR7 Regulates the Invasion, Angiogenesis and Tumor Growth of Human Hepatocellular Carcinoma Cells. *Journal of Experimental & Clinical Cancer Research*, **29**, Article No. 31. https://doi.org/10.1186/1756-9966-29-31

[42] Ramadan, F., Fahs, A., Ghayad, S.E. and Saab, R. (2020) Signaling Pathways in Rhabdomyosarcoma Invasion and Metastasis. *Cancer and Metastasis Reviews*, **39**, 287-301. https://doi.org/10.1007/s10555-020-09860-3

[43] Grymula, K., Tarnowski, M., Wysoczynski, M., *et al.* (2010) Overlapping and Distinct Role of CXCR7-SDF-1/ITAC and CXCR4-SDF-1 Axes in Regulating Metastatic Behavior of Human Rhabdomyosarcomas. *International Journal of Cancer*, **127**, 2554-2568. https://doi.org/10.1002/ijc.25245

[44] Würth, R., Bajetto, A., Harrison, J.K., Barbieri, F. and Florio, T. (2014) CXCL12 Modulation of CXCR4 and CXCR7 Activity in Human Glioblastoma Stem-Like Cells and Regulation of the Tumor Microenvironment. *Frontiers in Cellular Neuroscience*, **8**, 144. https://doi.org/10.3389/fncel.2014.00144

[45] Simpson, G., Relph, K., Harrington, K., Melcher, A. and Pandha, H. (2016) Cancer Immunotherapy via Combining Oncolytic Virotherapy with Chemotherapy: Recent Advances. *Oncolytic Virotherapy*, **5**, 1-13. https://doi.org/10.2147/OV.S66083

[46] Steckbeck, J.D., Deslouches, B. and Montelaro, R.C. (2014) Antimicrobial Peptides: New Drugs for Bad Bugs? *Expert Opinion on Biological Therapy*, **14**, 11-14. https://doi.org/10.1517/14712598.2013.844227

[47] Deslouches, B., Steckbeck, J.D., Craigo, J.K., Doi, Y., Burns, J.L. and Montelaro, R.C. (2015) Engineered Cationic Antimicrobial Peptides to Overcome Multidrug Resistance by ESKAPE Pathogens. *Antimicrobial Agents and Chemotherapy*, **59**, 1329-1333. https://doi.org/10.1128/AAC.03937-14

[48] Deslouches, B., Steckbeck, J.D., Craigo, J.K., Doi, Y., Mietzner, T.A. and Montelaro, R.C. (2013) Rational Design of Engineered Cationic Antimicrobial Peptides Consisting Exclusively of Arginine and Tryptophan, and Their Activity against Multi-

drug-Resistant Pathogens. *Antimicrobial Agents and Chemotherapy*, **57**, 2511-2521. https://doi.org/10.1128/AAC.02218-12

[49] Huang, L., Chen, D., Wang, L., *et al.* (2017) Dermaseptin-PH: A Novel Peptide with Antimicrobial and Anticancer Activities from the Skin Secretion of the South American Orange-Legged Leaf Frog, Pithecopus (Phyllomedusa) Hypochondrialis. *Molecules*, **22**, 1805. https://doi.org/10.3390/molecules22101805

[50] Mangoni, M.L., Papo, N., Saugar, J.M., *et al.* (2006) Effect of Natural L- to D-Amino Acid Conversion on the Organization, Membrane Binding, and Biological Function of the Antimicrobial Peptides Bombinins H. *Biochemistry*, **45**, 4266-4276. https://doi.org/10.1021/bi052150y

[51] Deslouches, B. and Peter Di, Y. (2017) Antimicrobial Peptides with Selective Anti-tumor Mechanisms: Prospect for Anticancer Applications. *Oncotarget*, **8**, 46635-46651.

[52] Papo, N., Shahar, M., Eisenbach, L. and Shai, Y. (2003) A Novel Lytic Peptide Composed of DL-Amino Acids Selectively Kills Cancer Cells in Culture and in Mice. *Journal of Biological Chemistry*, **278**, 21018-21023. https://doi.org/10.1074/jbc.M211204200

[53] Papo, N. and Shai, Y. (2003) New Lytic Peptides Based on the D,L-Amphipathic Helix Motif Preferentially Kill Tumor Cells Compared to Normal Cells. *Biochemistry*, **42**, 9346-9354. https://doi.org/10.1021/bi027212o

[54] Ellerby, H., Arap, W., Ellerby, L.M., *et al.* (1999) Anticancer Activity of Targeted Proapoptotic Peptides. *Nature Medicine*, **5**, 1032-1038. https://doi.org/10.1038/12469

[55] Chen, Y., Xu, X., Hong, S., *et al.* (2001) RGD-Tachyplesin Inhibits Tumor Growth. *Cancer Research*, **61**, 2434-2438.

[56] Papo, N., Braunstein, A., Eshhar, Z. and Shai, Y. (2004) Suppression of Human Prostate Tumor Growth in Mice by a Cytolytic D-, L-Amino Acid Peptide: Membrane Lysis, Increased Necrosis, and Inhibition of Prostate-Specific Antigen Secretion. *Cancer Research*, **64**, 5779-5786. https://doi.org/10.1158/0008-5472.CAN-04-1438

[57] Papo, N., Seger, D., Makovitzki, A., *et al.* (2006) Inhibition of Tumor Growth and Elimination of Multiple Metastases in Human Prostate and Breast Xenografts by Systemic Inoculation of a Host Defense-Like Lytic Peptide. *Cancer Research*, **66**, 5371-5378. https://doi.org/10.1158/0008-5472.CAN-05-4569

[58] Daly, J.W., Caceres, J., Moni, R.W., *et al.* (1992) Frog Secretions and Hunting Magic in the Upper Amazon: Identification of a Peptide That Interacts with an Adenosine Receptor. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10960-10963. https://doi.org/10.1073/pnas.89.22.10960

[59] Amiche, M., Ducancel, F., Mor, A., Boulain, J.C., Menez, A. and Nicolas, P. (1994) Precursors of Vertebrate Peptide Antibiotics Dermaseptin b and Adenoregulin Have Extensive Sequence Identities with Precursors of Opioid Peptides Dermorphin, Dermenkephalin, and Deltorphins. *Journal of Biological Chemistry*, **269**, 17847-17852. https://doi.org/10.1016/S0021-9258(17)32386-4

[60] Charpentier, S., Amiche, M., Mester, J., *et al.* (1998) Structure, Synthesis, and Molecular Cloning of Dermaseptins B, a Family of Skin Peptide Antibiotics. *Journal of Biological Chemistry*, **273**, 14690-14697. https://doi.org/10.1074/jbc.273.24.14690

[61] Van Zoggel, H., Hamma-Kourbali, Y., Galanth, C., *et al.* (2012) Antitumor and Angiostatic Peptides from Frog Skin Secretions. *Amino Acids*, **42**, 385-395. https://doi.org/10.1007/s00726-010-0815-9

[62] van Zoggel, H., Carpentier, G., Dos Santos, C., *et al.* (2012) Antitumor and Angiostatic Activities of the Antimicrobial Peptide Dermaseptin B2. *PLoS ONE*, **7**, e44351. https://doi.org/10.1371/journal.pone.0044351

[63] Opanda, S.M., Wamunyokoli, F., Khamadi, S., Coldren, R. and Bulimo, W.D. (2016) Genotyping of Enteroviruses Isolated in Kenya from Pediatric Patients Using Partial VP1 Region. *SpringerPlus*, **5**, Article No. 158. https://doi.org/10.1186/s40064-016-1834-0

[64] O'Brien, J., Wilson, I., Orton, T. and Pognan, F. (2000) Investigation of the Alamar Blue (Resazurin) Fluorescent Dye for the Assessment of Mammalian Cell Cytotoxicity. *European Journal of Biochemistry*, **267**, 5421-5426. https://doi.org/10.1046/j.1432-1327.2000.01606.x

[65] Rodríguez-Corrales, J. and Josan, J.S. (2017) Resazurin Live Cell Assay: Setup and Fine-Tuning for Reliable Cytotoxicity Results. In: Lazar, I., Kontoyianni, M. and Lazar, A., Eds., *Methods in Molecular Biology*, Vol. 1647, Humana Press, New York, 207-219. https://doi.org/10.1007/978-1-4939-7201-2_14

[66] Gavamukulya, Y., Maina, E.N., El-Shemy, H.A., *et al.* (2021) *Annona muricata* Silver Nanoparticles Exhibit Strong Anticancer Activities against Cervical and Prostate Adenocarcinomas through Regulation of CASP9 and the CXCL1/CXCR2 Genes Axis. *Tumor Biology*, **43**, 37-55. https://doi.org/10.3233/TUB-200058

[67] Abdelrahim, M., Baker, C.H., Abbruzzese, J.L., *et al.* (2007) Regulation of Vascular Endothelial Growth Factor Receptor-1 Expression by Specificity Proteins 1, 3, and 4 in Pancreatic Cancer Cells. *Cancer Research*, **67**, 3286-3294. https://doi.org/10.1158/0008-5472.CAN-06-3831

[68] Justus, C.R., Leffler, N., Ruiz-Echevarria, M. and Yang, L.V. (2014) *In Vitro* Cell Migration and Invasion Assays. *Journal of Visualized Experiments*, **88**, e51046. https://doi.org/10.3791/51046

[69] Liang, C.C., Park, A.Y. and Guan, J.L. (2007) *In Vitro* Scratch Assay: A Convenient and Inexpensive Method for Analysis of Cell Migration *in Vitro*. *Nature Protocols*, **2**, 329-333. https://doi.org/10.1038/nprot.2007.30

[70] Livak, K.J. and Schmittgen, T.D. (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods*, **25**, 402-408. https://doi.org/10.1006/meth.2001.1262

[71] Conlon, J.M., Woodhams, D.C., Raza, H., *et al.* (2007) Peptides with Differential Cytolytic Activity from Skin Secretions of the Lemur Leaf Frog *Hylomantis lemur* (Hylidae: Phyllomedusinae). *Toxicon*, **50**, 498-506. https://doi.org/10.1016/j.toxicon.2007.04.017

[72] Kramer, N., Walzl, A., Unger, C., *et al.* (2013) *In Vitro* Cell Migration and Invasion Assays. *Mutation Research/Reviews in Mutation Research*, **752**, 10-24. https://doi.org/10.1016/j.mrrev.2012.08.001

[73] Chen, H., Liu, H. and Qing, G. (2018) Targeting Oncogenic Myc as a Strategy for Cancer Treatment. *Signal Transduction and Targeted Therapy*, **3**, Article No. 5. https://doi.org/10.1038/s41392-018-0008-7

[74] Polakova, K., Polakova, K., Pizova, K., *et al.* (2015) *In Vitro* Cytotoxicity Analysis of Doxorubicin-Loaded/Superparamagnetic Iron Oxide Colloidal Nanoassemblies on MCF7 and NIH3T3 Cell Lines. *International Journal of Nanomedicine*, **10**, 949-961. https://doi.org/10.2147/IJN.S72590

[75] Katoh, M. (2016) FGFR Inhibitors: Effects on Cancer Cells, Tumor Microenvironment and Whole-Body Homeostasis (Review). *International Journal of Molecular Medicine*, **38**, 3-15. https://doi.org/10.3892/ijmm.2016.2620

[76] Libura, J., Drukala, J., Majka, M., *et al.* (2002) CXCR4-SDF-1 Signaling Is Active in Rhabdomyosarcoma Cells and Regulates Locomotion, Chemotaxis, and Adhesion. *Blood*, **100**, 2597-2606. https://doi.org/10.1182/blood-2002-01-0031

[77] Kucia, M., Reca, R., Miekus, K., *et al.* (2005) Trafficking of Normal Stem Cells and Metastasis of Cancer Stem Cells Involve Similar Mechanisms: Pivotal Role of the SDF-1-CXCR4 Axis. *Stem Cells*, **23**, 879-894. https://doi.org/10.1634/stemcells.2004-0342

[78] Jankowski, K., Kucia, M., Wysoczynski, M., *et al.* (2003) Both Hepatocyte Growth Factor (HGF) and Stromal-Derived Factor-1 Regulate the Metastatic Behavior of Human Rhabdomyosarcoma Cells, but only HGF Enhances Their Resistance to Radiochemotherapy. *Cancer Research*, **63**, 7926-7935.

Abbreviations

ARMS, Alveolar rhabdomyosarcoma; ATCC, American Type Culture Collection; CC_{50}, Cytotoxic Concentration; DrsB2, Dermaseptin B2; DOX, Doxorubicin; DMEM, Dulbecco's Modified Eagle's Medium; ERMS, Embryonal Rhabdomyosarcoma; FBS, Fetal Bovine Serum; FGFR1, Fibroblast growth factor 1; FNRMS, Fusion negative rhabdomyosarcoma; FPRMS, Fusion positive rhabdomyosarcoma; IC_{50}, Inhibitory Concentration; RMS, Rhabdomyosarcoma; SDF-1, SI, Selectivity Index; stromal derived growth factor.

Supplementary Materials

All the data analyzed is included in the manuscript.
https://data.mendeley.com/drafts/2mz6jw4zzc