Antitumoral activity against HeLa and MCF7 cells of two novel chimeric peptides derived from NF-κB inhibitor, CD21 binding region and antimicrobial peptides

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Trabajo de Grado para Optar el título de Químico

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Dedicatoria

A mi madre, quien fue mi apoyo, quien mantuvo constante este deseo y fue mi alegría en este proceso. A mi hermana, quien con paciencia y ternura estuvo ahí en cada momento y a su regalo del cielo, Tomás, quien sin palabras es capaz de trasmitirme toda su magia.

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Table of Contents

Lista de Tablas

Lista de Figuras

Resumen

TÍTULO: ACTIVIDAD ANTITUMORAL CONTRA CÉLULAS HeLa Y MCF7 DE DOS NUEVOS PÉPTIDOS QUIMÉRICOS DERIVADOS DEL INHIBIDOR DE NF-KB, DE LA REGIÓN DE UNIÓN CD21 Y DE PÉPTIDOS ANTIMICROBIANOS *

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PALABRAS CLAVES: PÉPTIDO QUIMÉRICO, CÁNCER, NF-KB, APOPTOSIS, CÉLULAS, RECEPTORES.

DESCRIPCIÓN:
Diferentes medicamentos que inducen la apoptosis de las células cancerosas a menudo no son selectivos y también afectan a las células normales. La inducción de apoptosis de un tipo celular específico en un organismo depende principalmente del reconocimiento de moléculas de superficie expresadas casi exclusivamente en este tipo de célula. Los receptores de células integrinas αvβ6 y CD21 podrían usarse para establecer una comunicación específica con células cancerosas porque están ausentes o son poco expresadas en células normales pero muy expresadas en algunos carcinomas, leucemias y linfomas. Se diseñó y sintetizó, 15 péptidos quiméricos para identificar células tumorales que expresan CD21 y la integrina αvβ6, utilizando los péptidos que se unen a CD21 o integrina αvβ6 o dos péptidos antimicrobianos unidos covalentemente al péptido del dominio de unión NEMO o al péptido mimético BCL-3 que inhibe la actividad de NF-κB. Los péptidos AM2-FN1, AM1 y FN2-MP9-Gp3, a 50 μM disminuyeron significativamente la viabilidad de las líneas celulares Hela y MCF7 pero no las células COS-7 y el péptido FN2 solo de la línea celular MCF7 como se determinó con ensayos de MTT y rojo neutro. La IC50 de AM2-FN1 y AM1 fue 16.82 y 27.17 μM para HeLa y 23.16 y 17.46 μM para MCF7. Los péptidos quiméricos, AM2-FN1 y FN2-MP9-Gp3, redujeron la viabilidad celular probablemente porque los péptidos NBD y BCL-3 impidieron la activación de la vía NF-κB desde el interior de la célula, considerando que los péptidos nativos no tenían este efecto. Los péptidos quiméricos, AM2-FN1 y FN2-MP9-Gp3 tienen potencial para ser utilizados como una alternativa quimiopreventiva y quimioterapéutica contra estos tipos de células tumorales.

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*Trabajo de Grado

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Abstract

TITLE: ANTITUMORAL ACTIVITY AGAINST HeLa AND MCF7 CELLS OF TWO NOVEL CHIMERIC PEPTIDES DERIVED FROM NF-KB INHIBITOR, CD21 BINDING REGION AND ANTIMICROBIAL PEPTIDES*

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KEYWORDS: CHIMERIC PEPTIDE, CANCER, NF-KB, APOPTOSIS, CELLS, RECEPTORS

DESCRIPTION:
Many drugs that induces apoptosis of cancer cell often are not selective and also affect normal cells. The induction of apoptosis of a specific cell type in an organism mostly depends on the recognition of surface molecules almost exclusively expressed in this cell type. The integrin αvβ6 and CD21 cell receptors could be used to establish a specific communication with cancer cells because they are absent or low expressed in normal cells but highly expressed in some carcinomas, leukemias and lymphomas. We designed and synthesized 15 chimeric peptides for targeting tumor cells expressing CD21 and the integrin αvβ6, using the CD21 - or integrin αvβ6 - binding peptides or two antimicrobial peptides covalently linked to either the NEMO binding domain peptide or the BCL-3 mimetic peptide that inhibits the NF-κB activity. Peptides AM2-FN1, AM1 and FN2-MP9-Gp3, at 50 μM significantly diminished the viability of Hela and MCF7 cell lines but not COS-7 cells and peptide FN2 only of MCF7 cell line as determined with neutral red and MTT assays. The IC50 of AM2-FN1 and AM1 was 16.82 and 27.17 μM for the HeLa and 23.16 and 17.46 μM for MCF7. Chimeric peptides, AM2-FN1 and FN2-MP9-Gp3, reduced the cell viability probably because the NBD and BCL-3 peptides prevented the NF-κB pathway activation from inside the cell considering that native peptides did not have this effect. Chimeric peptides, AM2-FN1 and FN2-MP9-Gp3 have potential to be used as a chemopreventive and chemotherapeutic alternative against these types of tumor cells.

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*Bachelor Thesis

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Introducción

Apoptosis is an essential process that cause the cell to activate an intrinsic suicide mechanism (J. F. R. Kerr, 1972; Kerr, 2002). There are alterations in the apoptotic pathways that transmit morphological and biochemical changes leading to cancer (Green and Evan, 2002). One of the mechanisms to eradicate tumor cell is the induction of apoptosis on these cells. Despite there are hundreds of molecules that are able of inducing apoptosis, most of them are not specific for tumor cells and affect also normal cells. The specific recognition of tumor cells using receptors expressed only or mainly in these cells and then trigger the apoptosis process is one of the strategies to attack mostly these tumor cells (Ricci et al., 2006). Once the specific molecules bind to the tumor cells, they could either get access to the cytoplasm where they can induce specifically the apoptosis of tumor cells or send the apoptotic trigger information from outside (Opferman and Korsmeyer, 2003).

Recent studies reveal that CD21 is overexpressed in chronic lymphocytic leukemia, pediatric follicular lymphoma and some carcinomas (Huang et al., 2013; Karnik et al., 2015; Michael et al., 1995). CD21 also known as CR2 belongs to the receptor family expressed in follicular dendritic and lymphoblastoid epithelial cells, mature B cells (Fischer and Kazatchkine, 2017; Karnik et al., 2015). CD21 is a single – chain integral transmembrane glycoprotein of 145 kDa that is the cellular receptor for human C3dg, C3d fragments and for Epstein Barr Virus through

the interaction of the gp350/220 (Ahearn and Fearon, 1989; Michael et al., 1995; Urquiza et al., 2005). The αvβ6 integrin is expressed mostly in epithelial cells but contrary to most epithelial integrin, is not expressed constitutively by healthy oral epithelial, being upregulated during tissue remodelling, including that accompanying wound healing and carcinogenesis (Niu and Li, 2017). Normally, the αvβ6 integrin binds to the ECM proteins fibronectin, vitronectin and tenascin, and also to the latency associated peptide of TGF-β1 (Thomas et al., 2006)There is increasing evidence indicating that avb6 promotes carcinoma progression by modulating invasion, inhibiting apoptosis, regulating protease expression and activating TGF-β1 (Bandyopadhyay and Raghavan, 2009; Thomas et al., 2006).

The nuclear factor kappa-B (NF-κB) regulates the genes involved in immunity, inflammation, cell proliferation, apoptosis and plays a key role in the development and progression of cancer (Echeverri R and Mockus S, 2008; Li and Verma, 2002). The NF-κB is the target for the development of new chemopreventive and chemotherapeutic agents because it regulates apoptosis (Karin, 2006). NF-κB is a family of five factors: p65/RealA, c-Rel, RelB, p50 and p52. The NF-κB p50 homodimer is stabilized through IκB, a protein of the BCL-3 family, avoiding the ubiquitination of the p50 subunit and in consequence the transcriptional activation of the NF-κB target genes. The interaction between p50 and BCL-3 regulates the inhibition or activation of the NF-κB. The binding regions of BCL-3 that interact with p50 have been identified and a peptide derived from these regions that inhibit the interaction between p50 and BCL-3 has been used to treat inflammatory diseases (Collins et al., 2015). Furthermore the NEMO- binding domain (NBD) peptide interferes the binding of NEMO (NF-κB essential modifier) to the IKK

complex resulting in the inhibition of the NF-κB dependent gene expression (May et al., 1989; Strickland and Ghosh, 2006).

Peptides are highly specific, often presented potent biological activity and offer more patient friendly treatments, resulting in an attractive powerful therapeutic option for many diseases (Hong et al., 2012). In this report, peptides 11382 and 11389, derived from the binding regions to CD21 of EBV gp350/220 involved in the invasion of the virus to B lymphocytes, peptide 11435 derived from the binding regions to integrin αvβ6 of EBV gp85/gH or two antimicrobial cationic peptides (AM1 and AM2) that interact with the cell membrane were covalently linked to NBD or to BCL-3 peptides and their in vitro effect on cell viability on HeLa, COS-7 and MCF7 was determined by MTT and Neutral Red assay.

MTT and neutral red assays were selected to determine the effect of these peptides on the cell viability. MTT assay is a colorimetric method for detecting living cells that can be used to measure cytotoxicity, proliferation or activation based on the conversion of MTT into insoluble formazan crystals (purple) by dehydrogenase (Huang et al., 2014; Zerbinati et al., 2018). On the other hand the neutral red uptake assay provides a quantitative estimation of the number of viable cells in a culture (Repetto et al., 2008), based on the ability of viable cells to incorporate and bind the neutral red into the lysosomes. This weakly cationic dye penetrates cell membranes by nonionic passive diffusion and concentrates in the lysosomes, where it binds by electrostatic hydrophobic bonds to anionic and/or phosphate groups of the lysosomal matrix (Repetto et al., 2008). Subsequently, the possible metabolic pathway of the cell death through cellular expression proteins apoptosis was evaluated by means of Western Blotting.

1. Materials and Methods

1.1 Peptide synthesis

Twenty eight peptides were synthesized. Chimeric peptides were formed by combinations between either peptides 11382, 11389 from CD21-binding regions of EBV gp350/220 (Urquiza et al., 2010, 2005), 11435 from the integrin αvβ6-binding regions of EBV gp85/gH (Urquiza et al., 2004), AM2 or AM1 (antibacterial peptides) and IKK-binding region of an amino-terminal α-helical region of NEMO (NF-κB essential modifier) that is called the NEMO binding domain (NBD) (May et al., 1989), was synthesized by the solid phase method with N-terminal amino acid protected with N -fluorenylmethoxycarbonyl (F-Moc) group obtained from Merck Novabiochem and the Rick-amide resin AM (100-200 mesh, substitution 0.58 mmol/g). the removal of the protective group was made with 20% piperidine in N,N- dimethylformamide (DMF) and carboxyl group activation was done with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF with N,N-Diisopropylethylamine (DIPEA) (Houghten, 1985). The peptide was acetylated to remove the amino charge using anhydrous acetic acid in dichloromethane (DCM) and peptides were cleaved by trifluoroacetic acid, triisopropylsilane (TIS), ethanedithiol and water (92.5: 2.5: 2.5: 2.5) during 2 hours. The peptides were precipitated using cold ether and after were lyophilized (Tam et al., 1983).

1.2 Peptides physicochemical characterization

Twenty eight peptides were analyzed by MALDI-TOF Ultraflextreme mass spectrometer (Bruker Daltonics, Bremen, Germany) operated by FlexControl Software (version 33 , Bruker Daltonics, Bremen, Germany) and equipped with a 355 nm Nd:YAG laser mass spectrometry on a matrix of α-cyano-4-hydroxycinnamic acid in ACN/H2O (1:1, 0.01% TFA) onto a ground steel target in a 1:1 ratio (sample: matrix). Four peptides were purity by RP-HPLC using a ZORBAX Eclipse XDB-C18 column (4.6 mm i.d. x 250 mm, 5µm, Agilent Technologies, USA) with an Agilent 110 system (Agilent Technologies, USA), eluted with a linear gradient of 30-100% v/v Acetonitrile (ACN) and solution H2O containing 0.1% TFA (v/v) over 30 min at flow rate 1 mL· min-1 and detection at 280 nm.

1.3 Cell line cultures

The human cervical cancer cell line HeLa , human mammary cancer cell line MCF7 and green monkey kidney cell line COS-7 was kept in culture EMEM supplemented with 8% fetal bovine serum containing 2 mM L-glutamine, 2,2 g/liter sodium bicarbonate, and 10 mM HEPES. All of the cells were grown at 37 ° C in a humidified atmosphere 5% CO2. Adherent cells, grown in monolayers were removed from culture box with 1000 µL trypsin - 0.53 mM EDTA. Adherent cell were counted in a Neubauer chamber according to cell lineage.

1.4 Cell Viability Assays

1 x10^4 adherent cells per well in 200 μL were seeded in 96-well flat-bottomed microplates at 37 °C and 5% CO_2, were incubated for 24 hours before peptide treatment. Twenty eight peptides at 50 μM in triplicate were added to HeLa, MCF7, COS-7 cells. (ATCC®, Manassas, VA). The cell viability was determined using MTT method after 48 hours of the incubation with these peptides (Mosmann, 1983). Based on these results, peptides were chosen to be tested with the viability assay using neutral red method that is more sensitive than the MTT method. In brief the assay was very similar to the MTT method, except that the peptide concentrations were 50, 30, 10, 1 y 0.3 μM (Fautz et al., 1991).

1.5 Statistical Analysis

Values were expressed as mean ± standard deviation (SD) in Viability Assays. Statistical significance One-way ANOVA followed by Tukey test was used for testing the difference between data. All analyses were performed using STATISTICA Software (version 8.0, StatSoft Inc., Tulsa, OK, USA). The $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) were considered to be statistically significant.

2. Results

2.1 Design of potential antitumoral Peptides

The chimeric peptides were designed to specifically bind to tumor cells and once they reach the cytoplasm to induce apoptosis. The tumor cell binding sequences were chosen from peptide 11382 and 11389 of EBV gp350/220 that bind to CD21 (Urquiza et al., 2005); from 11435 of EBV gp 85/gH that binds to the integrin αvβ6 (Urquiza et al., 2004), and from the cationic α-helix amphipathic peptides AM1 and AM2 that interact with the negative charge of the membrane (Bautista et al., 2018; Pérez Santamaría, 2015). The proapoptotic peptides selected were peptide FN1 that inhibits the complex formation of NEMO and IKK and prevents the nucleus NF-κB translocation and peptide FN2 from BCL-3 that interacts with p50 inhibiting the NF-κB activity (Collins et al., 2015; Strickland and Ghosh, 2006). The motif recognized by MMP9 was also selected having into account that often these tumor cells overexpress this enzyme (Chen, 2005). As a spacer sequences were used GG dipeptide. Chimerics peptides were designed containing a cell binding motif either in the amino or carboxi terminus covalently linked by peptide bond to NF-κB inhibiting peptide; sometimes depending on the length of these peptides in between these two peptides were added the GG dipeptide or the motif recognized by MMP9. Also the previously reported pro-apoptotic peptide AC1 and AC2 as a control (Miao et al., 2014; Weinreb et al., 2004). The predicted anti-cancer property for all the peptide sequences generated were calculated using the software AntiCP: Designing of Anticancer Peptides (Raghava et al., 2018) (Table 1). Only the peptides having a theoretical potential to be

antitumoral were selected to be synthetized having into account the large number of combinations that could be generated and the factibility of synthesis.

Table 1.

Native peptides and synthesized chimeric peptides

Number Assay	Peptide	MW (g/mol)	Primary Structure	Predited Anti-cancer	Source	Isoelectric point
1	FN1	1261,38	TALDWSWLQT	0.75	NBD	0
2	FN1.1	1390,5	TALDWSWLQTE	0.75	NBD	0
3	FN2	1735,08	AAVYRILSLFKLGSR	0.90	BCL-3	12.14
4	AC1	1948,17	GGLRRGDRPSLRYAMDS	0.31	*CDR	11.64
5	AC2	1982,36	FNFRLKAGAKIRFGRGC	0.80	*Apoptosis Control	12.42
6	AM1	2522.94	DLIWKLFSKAWEKFGKNKSR	0.80	*LFampin	11.21
7	AM2	1885.37	ATKKCALWSILKAVAKI	0.90	GAM019	11.15
	MP9	-	PRQITA	0.77	MMP9	-
8	Gp1	1563,65	FYSGNGPKASGGDYC	0.66	11389 gp350	5.92
9	Gp2	1682,9	HAEMQNPVYLIPET	0.56	11382 gp350	4.14
10	Gp2.2	1706,87	PETVPYIKWDNCNS	0.89	11382 gp350	3.93
11	Gp3	1938,34	EMQNLVYLILYKKKN	0.73	33220 *gp350	10.25
12	gH1	1239,34	KRVTEKGDEH	0.95	11435 gp 85	8.21
	gH2	2051.52	VFNLKDMFSRANRKRK	0.59	33210 *gp85	12.43
13	FN2-MP9	2016,43	VYRILSLFKLG-PRQITA	0.78	BCL-3 MMP-9	12.14
14	FN1-AM2	3201,78	TALDWSWLQT-GG-ATKKCALWSILKAVAKI	0.74	NBD GAM019	10.67
15	AM2-FN1	3201,78	ATKKCALWSILKAVAKI-GG-TALDWSWLQT	0.79	GAM019 NBD	10.67
16	FN2-AM2	3675,48	AAVYRILSLFKLGSR-GG-ATKKCALWSILKAVAKI	0.76	BCL-3 GAM019	11.64

17	FN1.1-Gp2	3014,32	TALDWSWLQTE-HAEMQNPVYLIPET	0.74	NBD 11382 gp350	3.54
18	FN2-MP9-Gp2	3640,26	VYRILSLFKLG-PRQITA-HAEMQNPVYLIPET	0.75	BCL-3 11382 gp350	9.95
19	FN1.1-Gp2.2	3038,3	TALDWSWLQTE-PETVPYIKWDNCNS	0.74	11382 gp350	3.43
20	FN2-MP9-Gp2.2	3664,24	VYRILSLFKLG-PRQITA-PETVPYIKWDNCNS	0.75	BCL-3 MMP-9 11382 gp350	9.87
21	FN2-MP9-Gp3	3895,7	VYRILSLFKLG-PRQITA-EMQNLVYLILYKKKN	0.75	BCL-3 33220 *gp350	10.83
22	FN1.1-Gp3	3269,77	TALDWSWLQTE-EMQNLVYLILYKKKN	0.74	NBD 33220 *gp350	7.09
23	FN2-MP9-gH2	4008,79	VYRILSLFKLG-PRQITA-VFNLKDMFSRANRKRK	0.75	BCL-3 MMP-9 33210 *gp85	12.31
24	FN1.1-gH2	3382,85	TALDWSWLQTE-VFNLKDMFSRANRKRK	0.74	NBD 33210 *gp85	11.35
25	FN1.1-Gp1	2895,08	TALDWSWLQTE-FYSGNGPKASGGDYC	0.74	NBD 11389 gp350	3.54
26	FN2-MP9-Gp1	3521,01	VYRILSLFKLG-PRQITA-FYSGNGPKASGGDYC	0.75	BCL-3 MMP-9 11389 gp350	10.13
27	FN1.1-gH1	2570,77	TALDWSWLQTE-KRVTEKGDEH	0.74	NBD 11435 gp85	4.59
28	FN2-MP9-gH1	3196,7	VYRILSLFKLG-PRQITA-KRVTEKGDEH	0.76	BCL-3 MMP-9 11435 gp85	10.93
	FN2-gH1*	2722.16	VYRILSLFKLG-GG-CRVTEKGDEHC	0.76	BCL-3 11435 pg 85	8.13

2.2 Peptides synthesis and physicochemical characterization

Twenty eight peptides were synthesized by the solid phase using to F-MOC strategy (Table 1). These peptides were characterized by HPLC to determine their purity, the mass determined in MALDI-TOF were compared to the expected mass. Those peptides containing many peptide species as determined by HPLC were purified on C18 column. In general, all these peptides presented purity >80% and the determined molecular mass was very similar to the expected molar mass.

2.3 Effect of these peptides on tumor Cell Viability using MTT Assay

The effect of these peptides at 50 μM on the viability of HeLa, MCF7 and COS-7 cells after 48 hours of incubation were determined by using the MTT method, measuring its conversion to formazan product whose absorbance is directly proportional to the number of metabolically active cells (Mosmann, 1983). The cell viability percentage in presence of the synthetic peptides during 48 hours was calculated and peptides decreasing the viability more than 50% were selected as potentially active tumor cell peptides. None of the native peptides affected the viability of COS-7 cells that was used as non-tumoral cells. The native peptides FN1, AM1, decreased the viability of HeLa and MCF7 cells for more than 50% and peptide FN2 decreased for >50% the viability of MCF7 (Figure 1). Four chimeric peptides, FN1.1-gH2, FN2-MP9-gH2, FN2-AM2 and FN1-AM2, decreased the viability of COS-7, HeLa and MCF7 in more than 50% and AM2-FN1 and FN2-MP9-Gp3 only in HeLa and MCF7 cell line. Peptide FN2-MP9-Gp2 promotes cell proliferation. The correlation of the effect of these peptides in the viability of these

three cell lines were 0.859 between HeLa and MCF7, 0.770 between HeLa and COS-7 and 0.788 between COS-7 and MCF7 (Figure 2).

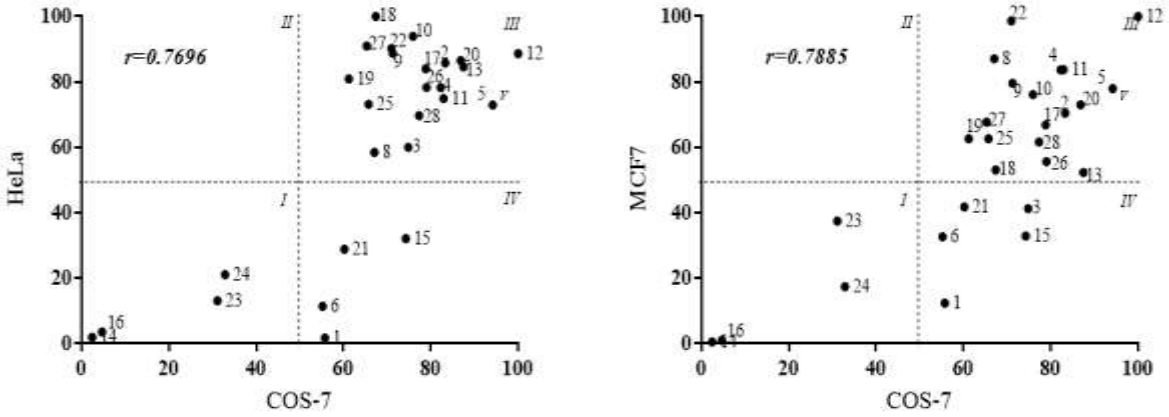

Figure 1. .Cell viability in cell treated with peptides in tumor cell line (HeLa- MCF7) and the control cell line (COS-7)

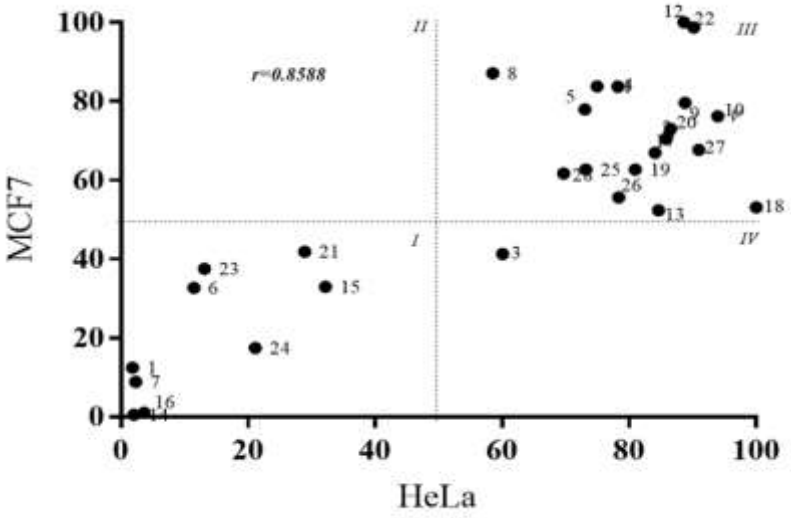

Figure 2.Comparison between cell viability in cell treated with peptides in tumor cell line (HeLa-MCF7)

2.4 MTT and Neutral Red cell viability assay in HeLa cell line

Neutral red and MTT assays determine the viable cells; however, Neutral Ned method has been reported to be more sensitive than MTT assay (Gomez Perez et al., 2017). The effect of these peptides at 50 μM on the cell viability at 24 hours determined with neutral red was compared with the effect at 48 hours determined with MTT and correlation analysis was performed. To facilitate the correlation analysis the data were normalized using the maximum and minimum values of viability (58 % viability with NR and 2.37 % viability with MTT). In general peptides that affect the viability at 48 hours also had the effect at 24 hours. The correlation of viability between 24 hours and 48 hours performed with all values, even with peptides that do not cause cell death at 24 hours, gave a r value of -0.465. Indeed, peptides were FN1, AM1, FN1-AM2, AM2-FN1, FN2-MP9-Gp3, FN2-MP9-gH2 and FN1.1-gH2 at 24 hour had a small effect on cell viability but at 48 hours the viability decreased for more than 70%, suggesting that these peptides had a slower kinetic than the other viability active peptides. Performing the correlation without these peptides FN1, AM1, FN1-AM2, AM2-FN1, FN2-MP9-Gp3, FN2-MP9-gH2 and FN1.1-gH2 the r value obtained was 0.661, with a 95% confidence interval of (0,1735 to 0,8886), indicating that the viability in presence of the other 21 peptides presented similar behaviour at 24 hours and at 48 hours (Figure 3).

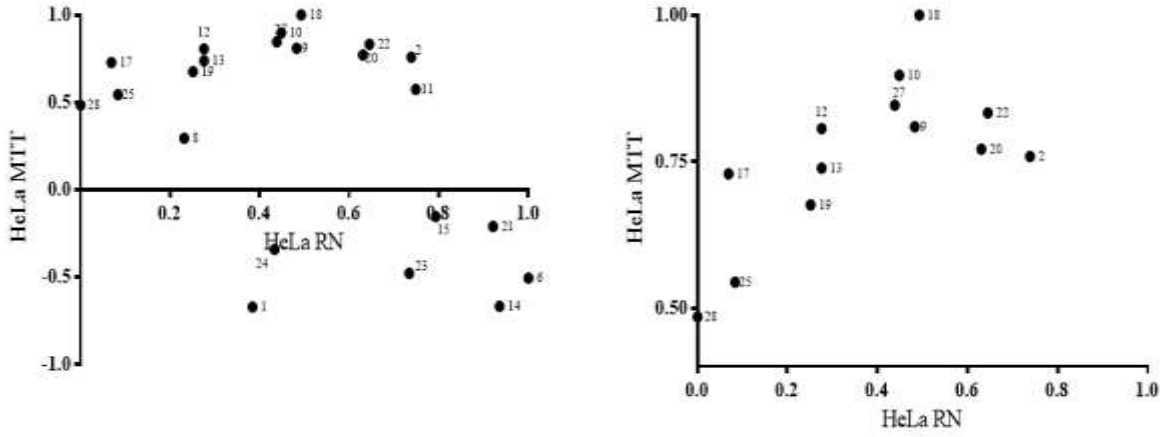

Figure 3.Comparison between MTT and NR cell viability assay in HeLa cell line

2.5 Determination of IC50 values of peptides affecting tumor cell Viability using the Neutral Red Method

To determine the IC50 of these peptides on the viability, the assay was performed at peptide concentrations varying from 0.3 to 50 μM during 48 hours in HeLa and MCF7. AM2-FN1 and AM1 was selected because showed statistically significant higher reduction on the viability ($p<0.001$) of the tumor cells (HeLa and MCF7) than on the control cell line (COS-7)(Figure 4). Both the NF-κB inhibitor peptide (NBD) FN1 and antimicrobial peptide AM2 that together form AM2-FN1 were also selected for this analysis. There was found that peptide AM2 presented the lowest IC50 and the peptide FN1 the highest IC50 in HeLa cell line, there were not significant differences among peptides FN1 y AM2 (Table 2).

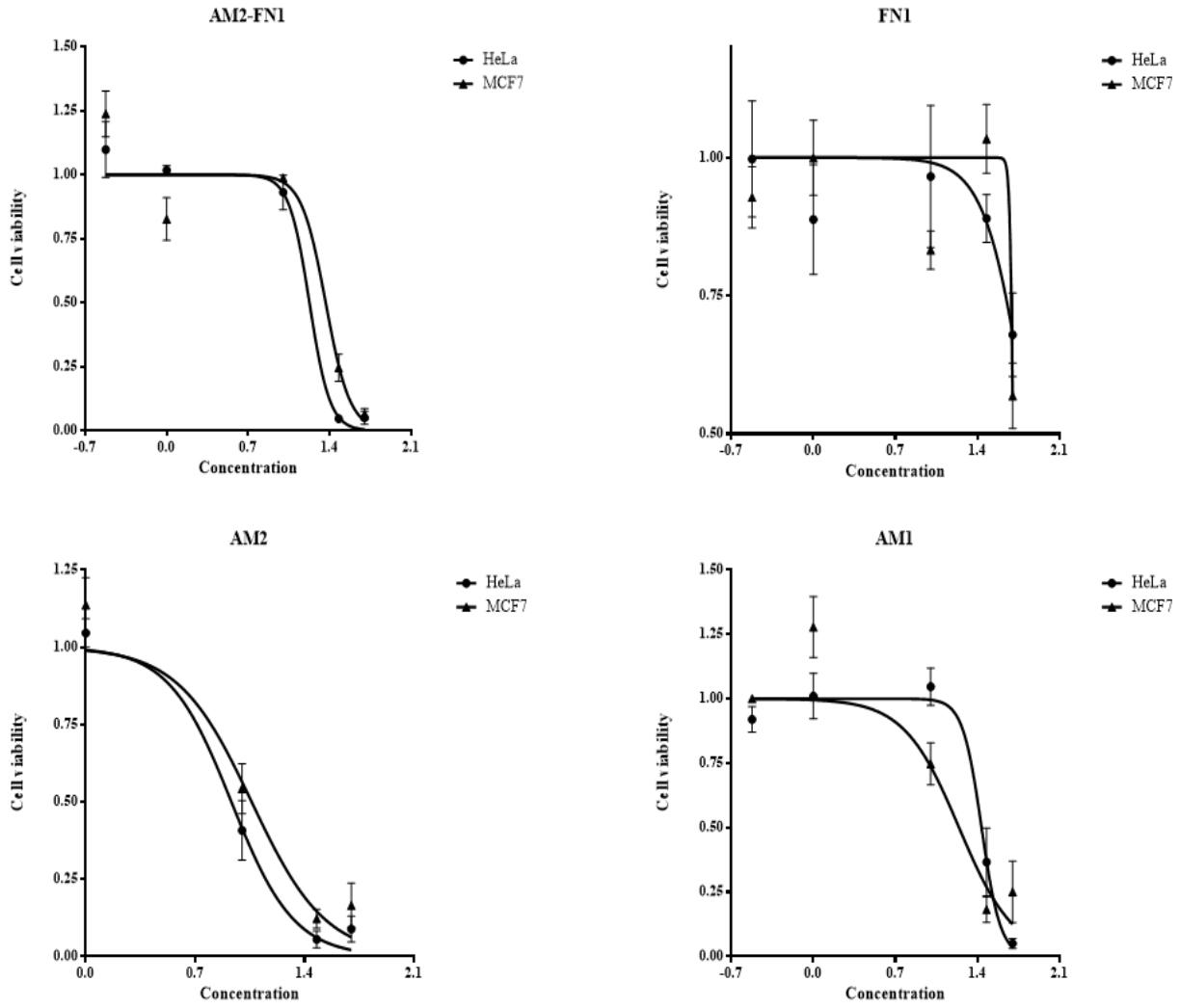

Figure 4.. Dose-Response curve between peptides concentration and cell viability of HeLa and MCF7 cells

Table 2.

IC50 of the peptides with the greatest effect in HeLa and MCF7 cell line

Peptide	**IC50**	
	HeLa	**MCF7**
AM2-FN1	16.82 ± 1.15	23.16±1.18
FN1	68.12±1.41	~50.46
AM2	8.53±1.24	11.51±1.44
AM1	27.17±1.11	17.46±1.20
FN2-MP9-Gp3*	26.89±8.76	39.87±13.81
FN2	ND	ND
Gp3	ND	ND

ND because did not inhibited affecting in μM

*was determinated based on MTT assay

3. Discussion

The chimeric peptides AM2-FN1 and FN2-MP9-Gp3 specifically reduced the viability of human tumor cell line HeLa and MCF7 but no the viability of COS cells. This biological effect was associated to the apoptosis induction, having into account the preliminary results of western blot. Interestingly, the effect was not present in the native peptides used to build these two chimeric peptides; specially the chimeric peptide that contains the binding region to CD21, the nemo peptide and in between the MMP9 recognition domain.

The AM2-FN1 chimera peptide decreased the activity of these tumor cells as determined with the MTT and neutral red test. The AM2-FN1 sequence ATKKCALWSILKAVAKI-GG-TALDWSWLQT, was built with the antimicrobial peptide AM2 and the NF-κB peptide

separated by two glycines that in theory allow both peptides move independently. The sequence TALDWSWLQT, from the peptide called NBD (NEMO-binding domain) is capable of blocking the NEMO association with the IKK complex which contains IKKα, IKKβ and the regulatory kinase IKKγ (NEMO); this peptide inhibits the activation of NF-κB because prevents the phosphorylation of IκB, which forms a complex with the NF-κB (Dai et al., 2004). The chimeric peptide could keep inactive the inhibitory protein IkB, (canonical NF-κB activation pathway), which in unstimulated conditions remains bound to NF-κB avoiding its translocation to the nucleus or modifying the catalytic activity of IKKα, which is necessary in the non-canonical activation of NF-κB in breast epithelial cells and B lymphocytes (Collins et al., 2015; Li and Verma, 2002; Lin et al., 1995). NF-κB is prevented from reaching the nucleus by modifying the activity of the inhibitory proteins due to the NBD peptide effect, generating the overexpression of target genes of cell progression and antiapoptotic genes.

The FN2-MP9-Gp3 peptide (VYRILSLFKLG-PRQITA-EMQNLVYLILYKKKN) affected the viability of HeLa and MCF7 cells despite peptides FN2, Gp3 and FN2-MP9 did not affect the viability of these cells by themself. Moreover, chimeric peptides FN1-AM2, FN2-AM2, FN2-MP9-gH2 and FN1.1-gH2 decreased the viability of the three cell lines despite no one native peptide had this activity. This suggests that the new characteristics of the some the chimeric peptide are the sum of the characteristics of the native peptides used to design these chimeric peptides; in other words the proapoptotic peptide alone was not able to affect the viability of these cells probably because the probability to go to the cytoplasm is lower than the probability of the chimeric containing both the binding and the proapoptotic peptides.

The viability of the cell lines incubated with the peptides FN1, AM1, FN1-AM2, AM2-FN1, FN2-MP9-Gp3, FN2-MP9-gH2 and FN1.1-gH2 during 24 hours was no significant different than the viability of non-treated cells but the viability of these cells compared to non-treated cells decreased significantly when the incubation the incubation with these peptides was during 48 hours. This indicates that the rate of these peptides to reduce these cell viability was lower than other tested peptides than could be due to the time spent to transport and process this chimeric peptide for releasing the FN2 peptide inside the cell for inhibiting the NF-κB signaling that result in the cell death.

The AM2-FN1 peptide presented an IC50 significant lower in HeLa than in MCF7 cell line, which was not found with native peptides FN1 and AM2 that were used to generate this chimeric peptide. There was significant differences in the effect on the viability between FN1 and FN1.1 despite the only difference between them was the additional glutamic residue at the carboxi terminus in peptide FN1.1; in fact FN1 significantly affect the viability of HeLa and MCF7 cells but FN1.1 did not have this effect on the viability of these cell lines. Probably the AM2 peptide facilitates the access to the cytoplasm of this chimeric peptide by binding to the cell membrane and could be detecting differences in the cell membrane between COS and HeLa or MCF7.

The AM1 peptide with primary structure DLIWKLFSKAWEKFGKNKSR, corresponds to a peptide derived from the amino acid residues 265-284 of bovine lactoferrin (LF) called lactoferrampin (LFampin) (Haney et al., 2009; Sinha et al., 2013; Van Der Kraan et al., 2004), which was modified by replacing the residue leucine (L) at position 271 by phenylalanine residue (F) and glutamine (Q) at position 275 by tryptophan (W) (Pérez Santamaría, 2015). This

peptide was included in this study because in previous studies it was identified that it possessed antimicrobial activity against SARM and E. coli O157:H7 and also has been reported to be active against tumor cells (Yang et al., 2002; Yoo et al., 1997).

4. Conclusion

Peptides FN1, AM1, AM2-FN1 and FN2-MP9-Gp3 presented a specific antiproliferative effect on HeLa and MCF7 cells, very likely associated to apoptosis activation. These peptides could be tested in other experiments to determine the potential to be used in animal model as chemopreventive agents against breast and cervical cancer. The results shown here support the use of chimeric peptides to get new biological activity not only apoptosis but also proliferation as was determined FN2-MP9-Gp2 peptide that caused survival signals in these cell lines. Four peptides decreased significantly the proliferation of these three cell lines and two chimeric peptides decreased the proliferation of HeLa and MCF7 cells.

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