Peptides 71 (2015) 170–178

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Anti-tumoral activity of human salivary peptides

João Pinto da Costa[a,1], Virginia Carvalhais[a,1], Francisco Amado[a], Artur Silva[b], Rita Nogueira-Ferreira[a], Rita Ferreira[a], Luísa Helguero[a,b,c,*], Rui Vitorino[a,c,*]

[a] Mass Spectrometry Centre, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[b] QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Institute for Biomedicine—iBiMED, Health Sciences Program, University of Aveiro, Portugal

ARTICLE INFO

Article history:
Received 12 February 2015
Received in revised form 15 July 2015
Accepted 15 July 2015
Available online 23 July 2015

Keywords:
Salivary peptides
Anti-tumoral activity
Human saliva

ABSTRACT

Chemotherapy continues to be the standard treatment for advanced or metastasized cancer. However, commonly used chemotherapeutic agents may induce damage in healthy cells and tissues. Thus, in recent years, there has been an increased focus on the development of new, efficient anticancer drugs exhibiting low toxicity and that are not affected by mechanisms of chemoresistance.

In the present work, we tested synthetic and naturally obtained human salivary peptides against breast, prostate, colon, osteosarcoma and bladder cancer cell lines (T47-D, PC-3, HT-29, MG63, T-24, respectively).

Results have showed that there is a reduced cell population increase that is peptide-, cell- and possibly pathway-specific, with the most potent effect observed in observed in T-47D breast cancer cells. Protein expression and microscopy results further indicate that, in this cell line, the peptide with the sequence GPPPQGGRPQG (GG peptide) interferes with the ability of cell adhesion proteins to stabilize adherens junctions, such as E-cadherin, leading to apoptosis. These promising results encourage future works aimed at disclosing the vast potential of salivary peptides as new therapeutic agents.

1. Introduction

Cancer retains high morbidity and mortality rates, despite the remarkable advances in treatment observed in recent year [12]. Although localized cancers may be successfully treated resorting to radiation therapy [18] and/or surgery [32], chemotherapy is the standard treatment for advanced or metastasized cancer. Nonetheless, commonly used chemotherapeutic agents, such as doxorubicin and cisplatin, inadvertently induce damage in healthy cells and tissues, resulting in numerous deleterious side-effects [16]. Additionally, some cancer cells may be quiescent, or show slow proliferation rate and, thus, are refractory to the cytotoxic effect of chemotherapeutic drugs acting at the DNA synthesis level [31]. Cancer cell adaptations that culminate in the increased expression of drug detoxifying enzymes and the activation of pro-survival pathways may also occur [21]. Consequently, there is an increasing demand need for the development of a new class of anti-cancer drugs that do not exhibit the toxicity of commonly used chemotherapeutic agents and that are not affected by mechanisms of chemoresistance.

In recent years, there has been an increasing interest in the anti-bacterial, anti-viral and anti-tumoral activities of a wide range of peptides [2,23,30]. These include synthetic peptides [20], naturally occurring peptides of the human immune system [35] and modified natural peptides [6]. In human saliva, there are numerous defense peptides that are involved in both innate and acquired immune response [14]. Although many of these molecules are present in low concentrations in the saliva, they often exert synergistic and/or cumulative effects, resulting in the rather efficient defense network of the oral cavity [15]. The salivary proteome is, however, quite complex, with more than 000 proteins already described [1]. Further research is still necessary for a better understanding of the mechanisms of action of salivary peptides as anti-microbial [11] and – possibly due to their immunomodulatory effects – as anti-tumoral agents.

Although research has blossomed aiming at determining the anti-tumoral properties and potential of antimicrobial peptides, these peptides are commonly obtained from multiple species, such as *Sus domesticus* (pig) [3], *Rana chensinensis* (Chinese brown frog)

* Corresponding authors at: Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.
E-mail addresses: luisa.helguero@ua.pt (L. Helguero), rvitorino@ua.pt (R. Vitorino).
[1] These authors contributed equally to this work

http://dx.doi.org/10.1016/j.peptides.2015.07.014
0196-9781/

Table 1
Sequence of the tested peptides.

Peptide	Amino acid sequence	Uniprot; AA position
GG	GPPPQGGRPQG	PRPC_HUMAN_131–150
KY	KRKFHEKHHSHRGY	HIS3_HUMAN_30–43
RQ	RFGYGYGPYQPVPEQPLYPQ	STAT_HUMAN_32–52
DR	DSSEEKFLR	STAT_HUMAN_20–28
Hist	DSHAKRHHGYKRKFHEKHHSHRGY	HIS3_HUMAN_20–43
F61	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPSRPPQ	PRB2_HUMAN_361–416
F64	MKFFVFALVLALMISMISADSHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN	HIS1_HUMAN

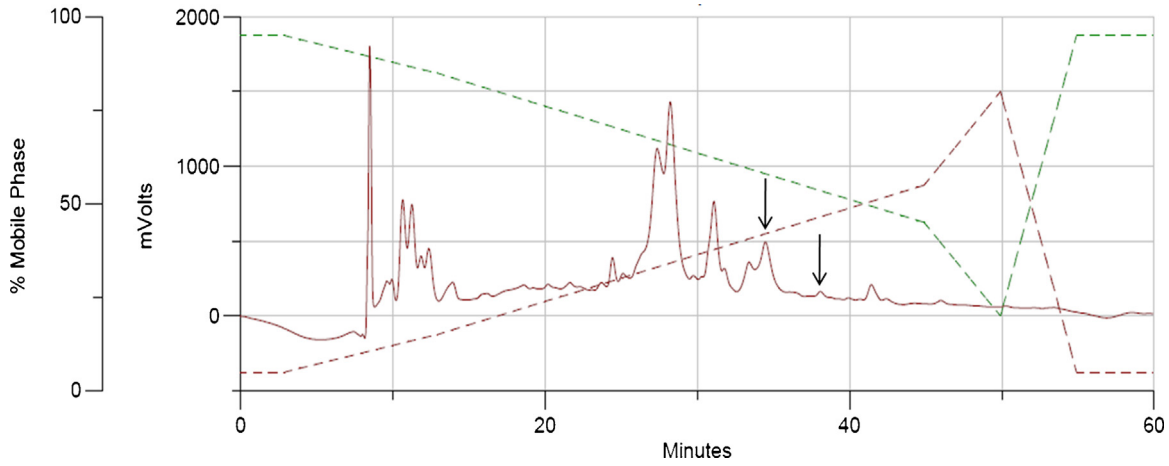

Fig. 1. RP-HPLC analysis of salivary proteins/peptides precipitated with TFA 0.2%. The gradient profile is shown. Arrows indicate the time points corresponding to the fractions chosen for subsequent studies, based on the MALDI-TOF/TOF analyses.

[33], *Xenopus laevis* (African clawed frog) [9], among others [22,28]. However, little research has been carried out on human antimicrobial peptides, with the exception of a handful of studies [26,37].

Thus, the aim of our work was to identify and isolate different human salivary peptides and test their potential anti-tumorigenic effect on a panel of cancer cell lines by analyzing how they affect cell proliferation, apoptosis and adhesion. These peptides showed different efficiency and selectivity which highlights the potential new trove of discoveries that lies within human salivary peptides and their possible use as therapeutic anti-tumoral agents.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN) was HPLC grade quality and acquired from VWR (Radnor, USA). All other general reagents, such as trifluoracetic acid (TFA), zinc chloride and α-cyano-hydroxycinnamic acid were purchased from Sigma–Aldrich (St. Louis, USA). Reagents for western blot were obtained from BioRad (Hercules, USA). RPMI 1640 medium, fetal bovine serum (FBS) and antibiotics were from Life Technologies (New York, USA). Anti-Bax (sc-493) and anti-AIF (apoptosis-inducing factor) (sc-13116) and anti-Bcl2 antibodies were from Santa Cruz Biotechnology (Texas, USA). Anti-cMyc (NB600-302) antibody was purchased from Novus Biologicals (Littleton, USA) and anti-caspase 3 cleaved from Calbiochem (Billerica, USA).

2.2. Peptides

2.2.1. Synthetic peptides

Synthetic peptides were obtained from Proteogenix (custom-made by ProteoGenix, France, >95% purity), dissolved in ultrapure water and kept at −80 °C until further use, yielding stock solutions of 1 mg mL^{-1}. Table 1 highlights the sequence of the peptides used in the present study. These were selected based on previous studies underlining their abundance in human saliva [8,15].

2.2.2. Salivary peptides

Salivary peptides were obtained as follows. Saliva was collected from multiple donors after their informed consent. All subjects showed good oral health and hygiene and did not exhibited signs of oral inflammations or other conditions. Additionally, saliva collections took place after a minimum of 30 min after the last meal. Whole saliva (WS) was collected by passive drooling and kept on ice. Then, WS was centrifuged at 14,000 × *g* for 30 min at 4 °C (Sigma 2–16KC). The supernatant was collected and kept at −80 °C until further use.

Proteins and peptides present in WS were purified using the TFA precipitation method described by Helmerhorst and collaborators [19]. Briefly, equal volumes of pooled saliva from multiple donors were mixed with TFA 0.2% (1:1) and magnetically stirred for 2 min. Thereafter, the precipitate was collected by centrifugation in the same conditions as mentioned above. Following centrifugation, the precipitate was freeze-dried and kept at −80 °C. Later, the freeze-dried sediments were weighted (Radwag 220/C/2) and, typically, 5 mg were dissolved in 1 mL of 2% TFA. Non-dissolved matter was removed by centrifugation, as described. Finally, supernatant was analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) on a preparative C-12 column connected to a Gilson 305HPLC device equipped with a UV–vis detector. Peptides were eluted at a flow rate of 1.5 mL min^{-1} and using a gradient generated from buffer A (0.1% TFA) and buffer B (80% acetonitrile, 0.1% TFA). The gradient consisted in the following steps: 0–3 min: 95% buffer A; 3–13 min: 95–85% buffer A; 13–45 min: 85–45% buffer A; 45–50 min: 45–20% buffer A; 50–55 min: 20–95% buffer A and 55–60 min: 95% buffer A. Absorbance was monitored at 219 nm and fractions were collected using an automated fraction collector with intervals of 30 s. Collected fractions were then dried in a speedvac (Thermo Savant SC210A) and re-suspended in ACN 50%