A Short SOX9 Peptide Mimics SOX9 Tumor Suppressor Activity and Is Sufficient to Inhibit Colon Cancer Cell Growth

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Abstract

Differently from cytotoxic chemotherapies, targeted therapies do not necessarily drive cancer cells toward death, but reduce cell proliferation, angiogenesis, and/or prevent metastasis without affecting healthy cells. Oncogenic proteins that are hyperactivated and/or overexpressed in cancer cells are prime targets for such therapies. On the other hand, the activity of tumor suppressor proteins is more difficult to harness. Here, we identified a short SOX9 sequence (S9pep) located at the hinge between the HMG DNA-binding domain and the SOX-E central conserved domain that mimics SOX9 tumor-suppressive properties. Doxycycline-induced S9pep expression in DLD-1 colorectal cancer cells inhibited the growth potential of these cells, including colorectal cancer stem cells, restored cell–cell contact inhibition, and inhibited the activity of the oncogenic Wnt/β-catenin signaling pathway. It also significantly decreased tumor growth in BALB/cAnNCrl mice grafted with mouse doxycycline-inducible CT26 colorectal cancer cells in which S9pep was induced by treating them with doxycycline. As the Wnt/β-catenin signaling pathway is constitutively activated in 80% of colorectal cancer and SOX9-inactivating mutations are present in up to 11% of colorectal cancer, S9pep could be a promising starting point for the development of a peptide-based therapeutic approach to restore a SOX9-like tumor suppressor function in colorectal cancer.

Introduction

Colorectal cancer is estimated to be the third most common cancer and the fourth cause of cancer-related death worldwide. The oncogenic Wnt/β-catenin signaling pathway is a prime target to fight colorectal cancer. Indeed, it is constitutively activated in inherited colorectal cancer (familial adenomatous polyposis) and in up to 80% of sporadic colorectal cancers, mostly due to inactivating mutations of the Adenomatosis polyposis coli (APC) tumor suppressor, a component of the β-catenin degradation complex. APC mutations are considered to be early and sufficient events to promote colorectal cancer development (1). Mutations in APC and in β-catenin, which are much less frequent (2, 3), result in a constitutive accumulation of β-catenin in the nucleus (3, 4) that is required for the formation of the active β-catenin/TCF transcriptional complex, leading then to the expression of pro-oncogenic Wnt/β-catenin signaling target genes, including cancer stem cells, restored cell–cell contact inhibition, and inhibited the activity of the oncogenic Wnt/β-catenin signaling pathway. It also significantly decreased tumor growth in BALB/cAnNCrl mice grafted with mouse doxycycline-inducible CT26 colorectal cancer cells in which S9pep was induced by treating them with doxycycline. As the Wnt/β-catenin signaling pathway is constitutively activated in 80% of colorectal cancer and SOX9-inactivating mutations are present in up to 11% of colorectal cancer, S9pep could be a promising starting point for the development of a peptide-based therapeutic approach to restore a SOX9-like tumor suppressor function in colorectal cancer.

SOX transcription factors are important modulators of the Wnt/β-catenin signaling pathway activity, as agonists or antagonists of the β-catenin/TCF complex, during development and disease (5). SOX9, the most extensively studied SOX family member in the intestine epithelium, is primarily expressed at the bottom of the crypts (i.e., in the stem/progenitor cell compartment) of small intestine and colon (6–9) and in the tuft cells along the villi of the small intestine (10). As the Wnt/β-catenin pathway is implicated in driving crypt epithelial cell proliferation (11), SOX9 expression pattern suggested possible interactions with factors of the Wnt/β-catenin signaling cascade, and a potential involvement in cell proliferation control. Indeed, SOX9 is both a downstream target and an inhibitor of the Wnt/β-catenin pathway (7, 12), as well as a critical actor in the control of cell proliferation, as indicated by the hyperplasia and dysplasia observed upon Sox9 knockout in the intestine epithelium (6). Recently, we reported SOX9 atypical tumor-suppressive activity (12, 13), a paradoxical finding when considering that SOX9 is overexpressed in colorectal cancer (14). A possible explanation to this surprising finding is the presence of SOX9-inactivating mutations in colorectal cancer (12, 15) and the aberrant expression of the SOX9 splice variant MinisoX9 (16) that could contribute to reduce SOX9 tumor suppressor activity, despite its high expression levels in colorectal cancer (13). Indeed, MinisoX9 not only behaves as a dominant negative SOX9 variant, but also stimulates the activity of the oncogenic Wnt/β-catenin pathway in colorectal cancer cells (16).

The known mechanisms underlying SOX9 regulation of Wnt/β-catenin signaling include gene expression, protein–protein interactions. 

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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were tested every two months for the absence of contamination using the MycoAlert Mycoplasma Detection Kit (Lonza). Native fluorescent peptides were synthesized by ProVpep/Synprosis and used at concentrations ranging from 0 to 1 mmol/L in DLD-1 cells.

Analysis of colorectal cancer cells and colonospheres

Cells and colonospheres were analyzed as described previously (12). Colorectal cancer cell clonogenic potential was evaluated by culturing 1,000 cells/10-cm dishes for 11 days and then counting the crystal violet (0.2% crystal violet; 2% ethanol)-stained colonies with the ImageJ software. For colorectal cancer cell growth evaluation by OD570 nm measurement (Polarstar BMG Labtech), 50 cells/well were plated in 96-well plates (12 wells per condition). At different time points (up to 10 days), they were stained with crystal violet and lysed in 1% SDS. Colonospheres were obtained by seeding 500 cells in 24-well ultra-low attachment plates (Costar, Thermo Fisher Scientific) with 500 μL of serum-free DMEM/F12 medium (Invitrogen) supplemented as described in ref. 12. Subcultures were obtained after centrifugation at 1,000 rpm for 5 minutes and dissociation to single cells using Accumax (Millipore). Cell viability was evaluated by using the nonradioactive CellTiter Cell Proliferation Assay (Promega).

Materials and Methods

Cells

DLD-1 CRC cells (ATCC) that express doxycycline-inducible Flag-S9pep with two nuclear localization signals (NLS) of the SV40 T-Antigen (sequence GATCCAAAAAAGAAGAAAGGTA) at the N-terminus (Flag-2NLS-S9pep; S9pep-DLD-1 cells) or any of the SOX9 peptides designed to better define the optimal sequence (accession number of the full-length SOX9 mRNA sequence: Z46629.1) were obtained by lentiviral infection using the pTRIPZ lentiviral vector (Dharmacon) in which the Turbo red fluorescent protein was substituted by the cDNA of interest, as reported in Blache and colleagues, 2004 (7). Transfection efficiencies were normalized

TopFlash luciferase assays were performed as in Blache and colleagues, 2004 (7). Transfection efficiencies were normalized
relative to the cotransfected phRG-TK standardization vector (Promega).

**qRT-PCR analysis**

Total RNA was isolated using the RNeasy Mini Kit (Qiagen), and then 1 µg was reverse transcribed with the SuperScript II Reverse Transcriptase kit (Invitrogen), as described previously (12). The SOX9 primers (i.e., forward CGAGGAAGTCCGGTGAG and reverse CTGGGATTGCCCCGAGTGCT) were described in Abdel-Samad and colleagues (2011; ref. 16); the c-Myc primers were those used in Prévostel and colleagues (2016; ref. 12) (Qiagen); the ALDH1 forward GTTGTCAAACCAGCAGCA and reverse CTGTAGGCCCATAACCAGGA primers were produced by Eurofins.

**Tumorigenesis model**

In vivo experiments were conducted as described in Prévostel and colleagues, 2016 (12) in agreement with the French Animal Ethics Committee (Agreement number 1152). Briefly, after intraperitoneal injection of $2 \times 10^5$ S9pep-CT26 cells, 6 weeks old BALB/AnN Crl mice (Charles River) were treated ($n = 8$) or not ($n = 8$) with 2 mg/mL doxycycline added in the drinking water. Tumor development was monitored by noninvasive bioluminescence imaging using a IVIS Lumina II camera (PerkinElmer), as described in Prévostel and colleagues, 2016 (12). Results were expressed as the average radiance (p/s/cm²/sr).

**3D models of S9pep, SOX9, and MiniSOX9**

The 3D structures adopted by the 2NLS-S9pep, SOX9, and MiniSOX9 sequences were predicted using the online Phyre2 software (http://www.sbg.bio.ic.ac.uk/phyre2/html). 3D models were visualized and manipulated with the online POLYVIEW-3D software (http://polyview.cchmc.org).

**Statistical analysis**

Statistical analyses were performed using GraphPad PRISM, version 5.0. Data represent the mean ± SEM. The Student t test was used to compare sets of data.

*Figure 1. Expression of a specific sequence within SOX9 (S9pep) is sufficient to increase colorectal cancer cell size and disrupt colorectal cancer cell morphology. A, Schematic view of the S9pep sequence (black box) position at the hinge between the HMG DNA-binding domain and the SOX-E central conserved domain of SOX9 and in the C-terminus of the tumor-associated SOX9 splice variant MiniSOX9. The S9pep is not conserved in the two other E-SOX members SOX8 and SOX10. B, Phase contrast images of S9pep-DLD-1 cells showing morphologic changes only in cells in which S9pep expression was induced by doxycycline (objective ×10); scale bar, 5 µm. C, Immunostaining of Sp9-DLD-1 cells incubated or not with doxycycline to induce S9pep expression. As expected, Flag-S9pep was localized in the nucleus due to the presence of two NLS sequence (Flag and Hoechst staining colocalization). Actin staining highlights the S9pep-induced increase of cell size and disruption of cell morphology (objective ×40); scale bar, 5 µm.*
Ethics approval

Animals housing and experiments were performed in accordance with the guidelines of the Institution for animal research (approval certificate D34-172-16) and were approved by the French animal ethics committee (Agreement number A34-454 for animals experimentations).

Availability of data and materials

The data and materials used in this study are available from the corresponding author on reasonable request.

Results

S9pep mimics SOX9-induced changes in colorectal cancer cells’ morphology

In a previous study, we showed that differently from what expected for a transcription factor, SOX9 can repress PKCα expression in intestine epithelial cells (23) through physical interaction of its central region (amino acids 208 to 303) with the SP1 transcription factor. This effect does not require SOX9 binding to DNA via its high mobility group (HMG) motif. Until then, no function had been attributed to SOX9 central region, which is highly conserved among SOX-E members (SOX8, SOX9, and SOX10; Fig. 1A). When coupled with two NLS, the flagged SOX9 (208-303) amino acid sequence alone prevented SOX9-induced PKCα repression in colorectal cancer cells and induced expression of PKCα (23), which has a tumor suppressor function in the intestine epithelium (24). However, none of the doxycycline-induced expression of flagged 2NLS-SOX9 (short sequences within 208-303) could induce PKCα expression when expressed in DLD-1 cells, suggesting that the entire SOX9 (208-303) sequence is required to control PKCα expression (Supplementary Fig. S1). Intriguingly, doxycycline-induced expression of the flagged 2NLS-SOX9 (208-229) sequence (S9pep), which is specific to SOX9 (Fig. 1A), correlated with phenotypic changes very similar to those we recently reported for full-length SOX9 (12). Specifically, S9pep accumulated in the nucleus of S9pep-DLD-1 cells, as expected (see Flag staining, Fig. 1C), and was clearly associated with an increase of cell size, diameter, and volume, as indicated by Scepter measurements (Supplementary Table S1), phase contrast images (Fig. 1B), and actin staining (Fig. 1C). This suggested that S9pep mimics the SOX9-induced phenotypic changes in colorectal cancer cells. All the related experiments/controls

Figure 2.

S9pep inhibits cell growth, decreases clonal capacity, and restores cell-cell contact inhibition in DLD-1 colorectal cancer cells. A, Cell growth of S9pep-DLD-1 cells incubated or not with doxycycline to induce S9pep expression was monitored by crystal violet staining (n = 12) at different time points during 18 days. B, The clonogenic potential of S9pep-DLD-1 cells is reduced in cells incubated with doxycycline to induce S9pep expression. Phase contrast images of crystal violet-stained S9pep-DLD-1 clones after 11 days in culture (starting from 1,000 isolated cells) in the presence or absence of doxycycline (objective ×10); scale bars, 100 μm. C, Quantification of the number of clones at day 11 (n = 3).
without or with doxycycline induction of SOX9 and MiniSOX9 performed in parallel with those reported here and in the following experiments are published in Prévostel and colleagues (2016; ref. 12).

Like SOX9, S9pep inhibits colorectal cancer cell growth and clonal capacity

As SOX9 inhibits the proliferation of intestine epithelial cells (6, 9) and colorectal cancer cells (12) and as S9pep mimics SOX9-induced phenotypic changes in colorectal cancer cells, we asked whether S9pep also could inhibit colorectal cancer cell growth. To this aim, we monitored the growth of S9pep-DLD-1 colonospheres in cultures exposed to doxycycline compared with control (no doxycycline; Fig. 2B and C). Doxycycline-induced S9pep expression also restored cell–cell contact inhibition, as shown by comparison of the phase contrast images of S9pep-DLD-1 cells incubated or not with doxycycline [Fig. 2B; the related experiments/controls without or with doxycycline induction of SOX9 and MiniSOX9 performed in parallel are published in Prévostel and colleagues (2016) (12)]. Moreover, qRT-PCR analysis of SOX9 and aldehyde dehydrogenase 1 (ALDH1), a cancer stem cell marker (26), showed that while SOX9 mRNA level was not affected, ALDH1 mRNA levels was drastically reduced upon doxycycline-induced S9pep expression (Fig. 3C). Together, these data suggest that S9pep is sufficient to inhibit colorectal cancer stem cell potential, and that this effect is not mediated through a variation of SOX9 expression or activity (see Supplementary Fig. S2 demonstrating no significant change in SOX9 transcriptional activity upon doxycycline-induced S9pep expression).

S9pep defines a new SOX9 functional domain that can inhibit the activity of the oncogenic Wnt/β-catenin signaling pathway and decrease c-Myc expression

SOX9 can be considered as a potent inhibitor of Wnt/β-catenin signaling and of c-Myc expression (12, 17), the master gene

**Figure 3.**

S9pep prevents colonosphere formation. A, Phase contrast images of S9pep-DLD-1 colonospheres cultured for 11 days in the absence or presence of doxycycline to induce S9pep expression (objective ×10); scale bars, 100 μm. B, CellTiter assay (n = 6) showing a decrease of cell viability in S9pep-DLD-1 colonospheres grown for 11 days in the presence of doxycycline compared with cultures without doxycycline. C, Quantitative RT-PCR analysis of SOX9 and ALDH1 mRNA expression in S9pep-DLD-1 colonosphere samples cultured for 11 days in the absence or presence of doxycycline to induce S9pep expression (n = 3) shows a significant decrease only in the level of the cancer stem cell marker ALDH1.
responsible for the oncogenicity of this pathway (27). As our findings indicated that S9pep retains several tumor suppressor properties of SOX9, we asked whether S9pep effects were related to inhibition of the activity of the Wnt/β-catenin signaling pathway, as reported for SOX9 (12, 17). TopFlash luciferase assays demonstrated that doxycycline-induced S9pep and SOX9 similarly inhibited Wnt/β-catenin signaling (Fig. 4A), including in cells that overexpress stabilized 33S-β-catenin (Fig. 4B). Moreover, Wnt/β-catenin signaling inhibition correlated with a significant reduction of c-Myc mRNA (Fig. 4C) and protein (Fig. 4C and D) levels. This demonstrates that S9pep functional properties are similar to those of SOX9 (12).

S9pep binds to β-catenin and relocates β-catenin from the chromatin to the cytosol

Accumulated evidences indicate that SOX9 inhibits Wnt/β-catenin signaling activity independently of its transcriptional activity. Indeed, MiniSOX9, which behaves as a dominant negative SOX9 variant (16), inhibits SOX9 transcriptional activity in a dose-dependent manner through direct competition with SOX9 for HMG-binding sites, but cannot prevent SOX9-induced Wnt/β-catenin signaling inhibition. Conversely, S9pep, which lacks the HMG DNA-binding domain, can inhibit Wnt/β-catenin signaling, like SOX9. Consistent with these observations, we recently reported that SOX9-induced Wnt/β-catenin signaling inhibition can be mediated through inhibition of β-catenin transcriptional activity, via a mechanism that involves the physical interaction of SOX9 with β-catenin and β-catenin relocation from the chromatin to the cytosol (12). Therefore, we asked whether S9pep inhibited Wnt/β-catenin signaling through this mechanism. A proximity ligation assay (Duolink), showed that, like SOX9 (12), S9pep physically interacted with β-catenin in doxycycline-treated S9pep-DLD-1 cells (Fig. 5A). Moreover, subcellular fractionation of S9pep-DLD1 cells demonstrated that S9pep expression decreased the fraction of chromatin-associated β-catenin (P3) and increased the cytosolic fraction of β-catenin (S1), compared with cells without doxycycline (Fig. 5B and C). Thus, the S9pep sequence is sufficient for the SOX9-mediated effect on β-catenin in colorectal cancer cells (12). Alignments of the S9pep sequence with the β-catenin–binding motifs in ICAT, APC, and E-cadherin highlighted a serine/threonine-rich sequence and a conserved aspartic acid within the N-terminus of all sequences, suggesting a pseudo homology. TopFlash assays (Fig. 5E) showed that deleting the final (S9k205G225) or the first 4 amino acids (S9k1213G231) of S9pep abolished the inhibitory effects on Wnt/β-catenin signaling. Conversely, mutation of the conserved aspartic acid into an alanine did not have any significant effect (see also Supplementary Fig. S3 providing similar conclusions with cell growth assays). Finally, removing only the last two amino acids in the C-terminus of S9pep (SOX9A206S227) slightly decreased the inhibitory effects of S9pep. This suggests that S9pep represents the optimal SOX9 sequence for inhibiting Wnt/β-catenin signaling. Importantly, removing one or both N-terminal NLS decreased S9pep-induced inhibition of S9pep.
DLD-1 cell growth (Supplementary Fig. S4), indicating that S9pep must be targeted into the nucleus to exert its tumor suppressor activity.

S9pep significantly reduces tumor development in vivo
The previous experiments indicated that S9pep retains SOX9 tumor suppressor properties in colorectal cancer cells (12) by reducing colorectal cancer cell/stem cell growth and by inhibiting the activity of the Wnt/β-catenin signaling pathway and the expression of c-Myc. As SOX9 overexpression prevents tumor growth in several in vivo models of primary tumors or metastasis (12), we asked whether S9pep also had antitumor effects in vivo. To this aim, we grafted mouse S9pep-CT26 colorectal cancer cells that constitutively express luciferase in the peritoneum of BALB/cAnNCrl mice (ref. 12; peritoneal carcinomatosis model), and assessed tumor development by bioluminescence at day 10 post-graft, on the basis of our previous findings using SOX9 (12).

The bioluminescent signal was significantly reduced in mice treated with doxycycline to induce S9pep expression (Fig. 6A and B), indicating a significant tumor growth inhibition by S9pep. Postmortem visual analysis confirmed the reduced tumor burden in mice treated with doxycycline to induce S9pep compared with controls (no doxycycline (Fig. 6C); the related experiments/controls without or with doxycycline induction of SOX9 and MinisoX9 performed in parallel are published in Prévostel and colleagues (2016) (12)). Together, these findings identify S9pep as a critical sequence for SOX9 antitumor activity and as a promising working basis for the development of peptide-based therapeutics against colorectal cancer.

Discussion
The Wnt/β-catenin signaling pathway is hyperactivated in 80% of colorectal cancer due to APC mutations that disconnect the
A SOX9 Peptide Inhibits the Oncogenic Wnt/β-Catenin Pathway

pathway from its Wnt extracellular signal and results in the constitutive intracellular accumulation of β-catenin, a key coactivator of TCF transcription factors that are the downstream transcriptional effectors of this pathway. β-Catenin is not only a transcriptional coactivator, but occupies a strategic position in the Wnt/β-catenin signaling, resulting in physical protein–protein interactions with both positive and negative regulators of this pathway. Thus, targeting these interactions is an attractive strategy for fighting colorectal cancer.

Among the inhibitors of the Wnt/β-catenin pathway that could be used for cancer therapy, agents that prevent the interaction of β-catenin with TCFs are receiving increasing interest for cancer drug development. Most of them are small molecules (28), but recent studies indicate that peptide-based approaches also can induce similar effects (29).

Here, we showed that S9pep, a 21-amino acid sequence located in SOX9 central region, inhibits Wnt/β-catenin signaling and the expression of c-Myc, its oncogenic target gene, by interacting with and relocating β-catenin from the chromatin to the cytosol, like previously described for full-length SOX9 (12). We also provide evidence that like SOX9, S9pep disrupts colorectal cancer cell morphology, reduces the growth of colorectal cancer cells and stem cells, and inhibits tumor development in vivo. Although the S9pep sequence is conserved in MiniSOX9, the tumor-associated SOX9 splice variant, MiniSOX9, does not interact with β-catenin.

**Figure 6.**
S9pep inhibits the growth of intraperitoneal colorectal cancer tumors. 
A, Bioluminescence imaging of tumor development at day 10 after intraperitoneal graft of S9pep-CT26 cells that constitutively express luciferase in mice that were treated or not with doxycycline (n = 8/group). 
B, Quantification of the bioluminescence signal (average radiance (p/s/cm²/sr)) resulting from tumor growth shows a reduced signal in mice treated with doxycycline to induce S9pep expression. 
C, Representative images of abdominal tumors in mice treated with doxycycline to induce expression of S9pep (right) or not (left).

**Figure 7.**
SOX9 (208–229) peptide in S9pep, SOX9, and MiniSOX9. The structural analysis of the conformation of the SOX9 (208–229) peptide sequence (in red) within S9pep (A), SOX9 (B), and MiniSOX9 (C).
and does not have tumor suppressor activities (12). This suggests that within MiniSOX9, the S9pep sequence might not be properly exposed and/or folded and thus cannot interact with β-catenin. This could be caused by the close vicinity of the S9pep sequence to the MiniSOX9 DNA binding HMG domain that might hide and/or constraint S9pep (Fig. 7 and see the following website links for animated views of SOX9(208–229) within S9pep (http://polyview.cchmc.org/cgi-bin/gallery.cgi?Task=entry&Lib=3Da&IMG=15305693385613), SOX9(http://polyview.cchmc.org/cgi-bin/gallery.cgi?Task=entry&Lib=3Da&IMG=15305696723733), and MiniSOX9 (http://polyview.cchmc.org/cgi-bin/gallery.cgi?Task=entry&Lib=3Da&IMG=15305678965751).

Given its antitumor properties and reasonably small size suitable for the production of synthetic peptides, S9pep can be viewed as a relevant working basis for the development of a peptide-based therapeutic approach for colorectal cancer. Indeed, besides the promising clinical outcomes for cancer diagnosis and prognosis, novel peptide therapies for patients with cancer, including colorectal cancer, have emerged in recent years (30). The intrinsic properties of peptides (i.e., high selectivity, good efficiency, relative safety, and good tolerability) greatly contributed to their success. However, the peptides currently used for cancer treatment are mainly against specific extracellular targets on tumor cells, for regulating tumor cell biosynthesis, delivering drugs in tumor cells, such as peptide-based chemotherapies (30), or inducing specific T-cell responses against tumor cells. Conversely, the therapeutic use of peptides to target intracellular signals is considered less attractive because native peptides do not generally cross the cell membrane. Another limitation is that natural peptides usually have a relatively short plasma half-life due to enzymatic degradation. Unfortunately, a native synthetic S9pep coupled to a FITC fluorophore also is almost completely degraded after one day of incubation in cell culture medium (mass spectrometry analysis) and cannot penetrate into DLD-1 colorectal cancer cells because no fluorescent signal was detected in DLD-1 cells when FITC-S9pep was used. Therefore, we need to optimize S9pep cell penetration capacity and stability, as recently done for peptidomimetics used for targeting β-catenin–dependent signaling in chondrocytes and cartilage (31). The chemical technologies that have emerged in recent years (e.g., coupling to cell penetration peptides, amino acid substitutions, enhancement of the secondary structure, coupling to vehicles, attachment to polyethylene glycol chains—reviewed in Fosgerau and colleagues (2015) (32)) will certainly be of help for improving S9pep half-life and bioavailability.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: P. Blache, G. Subra, C. Prevostel Development of methodology: P. Blache, C. Prevostel Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P. Blache, L. Caniter-Thouennon, M. Busson, C. Prevostel Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Blache, C. Prevostel Writing, review, and/or revision of the manuscript: P. Blache, L. Caniter-Thouennon, M. Ychou, C. Prevostel Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Verde, M. Ychou Study supervision: C. Prevostel Other (peptide synthesis strategies): G. Subra

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