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ScienceDirect
Acta Biomaterialia 5 (2009) 842–853
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Inhibition of cancer cell proliferation by designed peptide amphiphiles ☆

Lorenzo Aulisa [a], Nico Forraz [b], Colin McGuckin [b], Jeffrey D. Hartgerink [a,*]

[a] Departments of Chemistry and Bioengineering, Rice University, Houston, TX 77005, USA
[b] Centre for Cord Blood, School of Clinical and Laboratory Sciences, North East England Stem Cell Institute, Institute of Human Genetics, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Received 15 May 2008; received in revised form 5 November 2008; accepted 6 November 2008
Available online 27 November 2008

Abstract

HOX genes encode conserved transcription factors that control the morphological diversification along the anteroposterior body axis. HOX proteins bind to DNA through a highly conserved 60 amino acid sequence called the homeodomain, and greater DNA binding specificity and stability are achieved when it forms complexes with cofactors such as PBX and MEIS in humans. In particular, HOX proteins from paralog groups 1–8, interact with PBX proteins via a specific and highly conserved hydrophobic six amino acid sequence localized in the N-terminal region of HOX. In several oncogenic transformations, deregulated HOX gene expression has been observed, indicating an involvement of these transcriptional regulators in carcinogenesis and metastasis. Inhibition of the HOX–PBX interaction could be a strategy to control the abnormal proliferation of these cancer cells. In this study we describe a small designed peptide amphiphile (PA) which self-assembles into micelles and shows inhibition of T3M4 pancreatic cancer cells, K562 leukemia cells and MJT1 melanoma cells while non-cancerous fibroblast NIH 3T3 cells are less affected. This molecule contains three critical regions: a 9-amino-acid sequence designed to disrupt HOX/PBX/DNA complex formation, a 16-amino-acid sequence to deliver the peptide into the cell and a 16-carbon-acyl chain which we show leads to the molecule's self-assembly and significantly enhances the effectiveness of the molecule to slow cell proliferation.

Keywords: HOX; PBX; Pancreatic cancer; Peptide amphiphile; Hexapeptide mimic

1. Introduction

At least 200 homeobox genes exist in the human genome, encoding a variety of transcription factors involved in morphogenesis and homeostasis [1]. All these genes are characterized by a nucleotide sequence (the homeobox) that code a 60 amino acid helix-turn-helix DNA binding motif (the homeodomain), which is highly conserved between species [2–4]. Homeobox genes are divided into several families based on the similarity of their homeodomains. HOX genes, a subset of this family, are organized into four clusters (A–D), and in each cluster there are 8–11 genes localized on different chromosomes [4–7]. To date, 39 HOX genes have been discovered in the human genome [4,5,8–10]. HOX genes control the body formation and segmentation during vertebrate and invertebrate embryogenesis, and are also important in the regulation of primitive and adult hematopoiesis [6,8,9,11]. Abnormal HOX gene expression has been reported in primary solid tumors and in many hematological malignancies [12]. For example, HOX A1 [13,14], silent in normal mammary

Abbreviations: AcOH, acetic acid; CD, circular dichroism; DAPI, 4′-6-diamidino-2-phenylindole; DCM, dichloromethane; DIC, *N*,*N*′-diisopropylcarbodiimide; DIEA, diisopropylethylamine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; FMOC, flourenylmethyloxycarbonyl; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate; HoBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TFA, triflouroacetic acid; TFE, trifluoroethanol

☆ Part of the Self-Assembling Biomaterials Special Issue, edited by William L. Murphy and Joel H. Collier.

* Corresponding author. Tel.: +1 713 348 4142; fax: +1 713 348 4201.
E-mail address: jdh@rice.edu (J.D. Hartgerink).

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doi:10.1016/j.actbio.2008.11.002

gland, has been found active in breast cancers [15]; HOX B7, silent in normal melanocytes, is active in melanomas, where it is found to be responsible for an overproduction of bFGF [1,16,17]. Therefore, modulation of the activity of HOX proteins could be a strategy to control the behavior of cancer cells [18].

Several classes of non-HOX-homeodomain-containing genes, like PBX, have been shown to work as cofactors with HOX protein [19]. PBX belongs to a family of transcription factors called TALE (Three-Amino acids-*L*oop-*E*xtension between helix 1 and helix 2) [5,11,20–22]. They give DNA binding specificity to HOX proteins belonging to paralog groups from 1 to 8 [19], when they form heterodimers. This binding interaction is mediated through a specific and highly conserved hydrophobic hexapeptide [5,11,23–28]. The interaction of HOX with PBX alters the selection of DNA binding sites and in some cases may change the way in which the transcription is regulated [29,30]. These six amino acids are localized outside the homeodomain and are connected to it through a linker arm of variable length. In this motif, tryptophan and methionine are present for all HOX gene products of paralogs 1–8. Crystal structures of this complex [11,25] show that tryptophan is able to enter in a binding pocket formed by PBX from the TALE and the C-terminal end of helix 3. The interaction with PBX is mediated by an N-terminal motif where asparagine and tryptophan are conserved [19,28].

Several groups have shown that synthetic peptides which mimic the hexapeptide motif can interfere with HOX/PBX binding [2,10,31–33]. However, to disrupt HOX/PBX binding the peptide must be able to enter the cell. Our study had two major goals: to determine the benefits of (i) adding a cell penetrating peptide sequence to the hexapeptide motif and (ii) adding a hydrophobic domain to the peptide via a hydrophobic alkyl chain to initiate self-assembly [34–37]. These additions to the hexapeptide motif were assessed primarily though proliferation studies on T3M4 pancreatic cancer cells. The best peptide–drug candidates were also tested on K562 leukemia cells, MJT1 melanoma cells and NIH 3T3 fibroblast cells.

The common architecture of a peptide amphiphile consists of two regions having opposite chemical properties. There is a hydrophobic region, which in this case consists of an alkyl tail with 16 carbon atoms coupled to the amino terminus of the peptide. The second region is the hydrophilic peptide. Together this makes an amphiphilic molecule. Above its critical micelle concentration, the peptide amphiphile can potentially be organized into different structures, including spheres and fibers, depending on the molecular design and the concentration at which the peptide amphiphile is used. At high concentration, and with only one alkyl tail as hydrophobic region, they are usually organized in nanofibers with a diameter of 7.6 ± 1 nm [34]. These nanofibers have been shown to have wide application in biomaterials and tissue engineering fields, as recently reported in bone regeneration and other tissue engineering approaches [37–41]. In this work we have used the peptide amphiphile architecture, but at lower concentrations, in order to assemble spherical micelles.

Our peptide–drug molecule is designed to mimic the hexapeptide motif [42] and therefore block HOX/PBX complex formation. T3M4 pancreatic cancer cells were chosen as a target because pancreatic cancer is particularly aggressive, with a 5-year survival rate of less than 10%. Currently, surgical treatment is the only viable option, but only 10–20% of patients are suitable for this procedure [43]. Furthermore, in pancreatic cancer, several HOX proteins are found to be de-regulated, in particular HOXB2, which is not expressed in the normal pancreas. Survival after surgery was found to be significantly longer for patients with no HOXB2 expression [44,45]. Therefore, it has previously been proposed that approaches which can disrupt the HOX/PBX complex formation may offer an important alternative or combined therapy for the treatment of pancreatic cancer or other forms of cancer in which HOX/PBX deregulation is involved [10,33,46].

2. Materials and methods

2.1. Cells and culture medium

T3M4, MJT1 and K562 cells were cultured in RPMI medium, while NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium; both media were supplemented with 10 vol.% fetal bovine serum (FBS) and 1 vol.% antibiotic–antimycotic (penicillin G sodium, streptomycin sulfate, amphotericin B), all provided by GIBCO–Invitrogen Corporation. The phosphate-buffered saline solution (PBS) and trypsin–EDTA (1×) were provided by GIBCO. The 75 cm^2 tissue culture flasks with a 0.2 μm vent cap, the 25 cm^2 tissue culture flasks, and the 6- and 24-well tissue culture plates were purchased by Fisher Scientific. Trypan blue solution (0.4%) was purchased from Sigma–Aldrich. Cells were counted using a hemocytometer provided by Hausser Scientific.

The cryovial containing the cells were removed from liquid nitrogen storage container and left in a water bath for a few minutes until the cells were completely defrosted. The cells were quickly removed and transferred to a 15 ml conical tube, and 10 ml of fresh medium (37 °C) was added in order to dilute the dimethyl sulfoxide (DMSO). The tube was spun for 6 min at 1300*g*. The supernatant was removed from the tube and the pellet of cells was suspended in 1 ml of fresh medium. Then 100 μl of this suspension was transferred to a 75 cm^2 tissue culture flask for T3M4, MJT1 and NIH 3T3 cells, or to a 25 cm^2 tissue culture flask for K562 cells, with fresh medium. The cells were placed in a 37 °C incubator with 5% CO_2. In the case of adherents cells, their density was monitored daily and the culture was passaged when the confluency was approximately 80%.

ID	Title	Pages
2221	Inhibition of cancer cell proliferation by designed peptide amphiphiles ☆	12