ELSEVIER

Cell Biology International 32 (2008) 1467–1477

Cell Biology International

www.elsevier.com/locate/cellbi

Effect of polyamine deficiency on proteins involved in Okazaki fragment maturation

Veronica M. Johansson [a,*], Maria Falck Miniotis [a], Cecilia Hegardt [b], Göran Jönsson [b], Johan Staaf [b], Pia S.H. Berntsson [a], Stina M. Oredsson [a], Kersti Alm [a]

[a] *Department of Cell and Organism Biology, Lund University, Helgonavägen 3B, SE-223 62 Lund, Sweden*
[b] *Department of Oncology, Clinical Sciences, Lund University, Lund, Sweden*

Abstract

Polyamine depletion causes S phase prolongation, and earlier studies indicate that the elongation step of DNA replication is affected. This led us to investigate the effects of polyamine depletion on enzymes crucial for Okazaki fragment maturation in the two breast cancer cell lines MCF-7 and L56Br-C1. In MCF-7 cells, treatment with N^1,N^{11}-diethylnorspermine (DENSPM) causes S phase prolongation. In L56Br-C1 cells the prolongation is followed by massive apoptosis. In the present study we show that L56Br-C1 cells have substantially lower basal expressions of two Okazaki fragment maturation key proteins, DNA ligase I and FEN1, than MCF-7 cells. Thus, these two proteins might be promising markers for prediction of polyamine depletion sensitivity, something that can be useful for cancer treatment with polyamine analogues. DENSPM treatment affects the cellular distribution of FEN1 in L56Br-C1 cells, but not in MCF-7 cells, implying that FEN1 is affected by or involved in DENSPM-induced apoptosis.

Keywords: DNA ligase I; FEN1; human breast cancer; DENSPM; CGP 48664

1. Introduction

DNA is replicated simultaneously at the two template strands in eukaryotic cells. The leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously. The discontinuous synthesis on the lagging strand is achieved by the joining of multiple short DNA fragments, around 100–150 nucleotides long, designated Okazaki fragments. The situation on the lagging strand is far more complex than that on the leading strand and at least 23 different proteins are involved (Hubscher and Seo, 2001). The replication process on the lagging strand is initiated with the synthesis of an RNA primer, which is extended with a DNA primer by DNA polymerase α (pol α). Since pol α is incapable of proofreading, the primer must later be replaced (MacNeill, 2001). Further elongation takes place when the clamp loader replication factor C (RF-C) displaces pol α and loads the sliding clamp proliferating cell nuclear antigen (PCNA). DNA polymerase δ (pol δ) binds to RF-C and PCNA and is responsible for DNA synthesis. When pol δ reaches the 5′-end of the next Okazaki fragment, a flap is formed by displacement of the segment containing the RNA primer together with the DNA primer synthesized by pol α. There are several proposed models of how this is accomplished. According to one model, DNA2 removes the RNA primer and Flap endonuclease 1 (FEN1) then removes the DNA synthesized by pol

Abbreviations: AdoMetDC, *S*-adenosylmethionine decarboxylase; BASE, BioArray Software Environment; BAC, bacterial artificial chromosome; CGP 48664, 4-amidinoindan-1-one 2′-amidinohydrazone; DENSPM, N^1,N^{11}-diethylnorspermine; ECL, enhanced chemiluminescence; FCM, flow cytometry; FCS, fetal calf serum; FEN1, flap endonuclease 1; HRP, horseradish peroxidase; NIM, nuclear isolation medium; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline with Tween 20; PCNA, proliferating cell nuclear antigen; PI, propidium iodide; pol α, DNA polymerase α; pol δ, DNA polymerase δ; RF-C, replication factor C; RNase A, ribonuclease A type II; SSAT, spermidine/spermine N^1-acetyltransferase.

* Corresponding author. Tel.: +46 46 222 8201; fax: +46 46 222 4539.
E-mail address: veronica.johansson@cob.lu.se (V.M. Johansson).

1065-6995/$ - see front matter © 2008 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.cellbi.2008.08.018

α (Bae et al., 2001, Kao and Bambara, 2003). In another model, the FEN1-only model, FEN1 alone removes the RNA as well as the DNA synthesized by pol α (Kao and Bambara, 2003). In a third model, it is suggested that the enzyme RNase H removes most of the RNA primer and then FEN1 steps in and removes residues of the RNA primer and the DNA synthesized by pol α (Kao and Bambara, 2003). In all proposed models of Okazaki fragment maturation, the nick is sealed by the enzyme DNA ligase I (Kao and Bambara, 2003, MacNeill, 2001). PCNA has been shown to stimulate both DNA ligase I activity (Tom et al., 2001) and FEN1 activity (Li et al., 1995, Wu et al., 1996).

We have previously shown that one of the early effects of polyamine pool depletion is a prolongation of the S phase (Alm et al., 2000), and our studies indicate that it is the elongation step of DNA synthesis, as opposed to the initiation step, that is affected (Alm and Oredsson, 2000). Our previous research has shown that the activity of topoisomerase II is lowered in polyamine deficient cells (Alm et al., 1999), something that probably is involved in the S phase prolongation. Polyamines are positively charged at physiological pH and bind to negatively charged structures in the cell, such as DNA, RNA and proteins. Most of these electrostatic interactions are specific and the polyamines can not be replaced by other cations (Thomas and Thomas, 2003). Lack of polyamines is known to affect the chromatin structure and renders cells more sensitive to DNA damaging agents such as radiation (Williams et al., 1994). It has been shown that polyamine deficient cells accumulate Okazaki-like fragments (Pohjanpelto and Holtta, 1996), something which might be due to an incomplete or inefficient Okazaki fragment maturation process.

The polyamines, putrescine, spermidine and spermine, are present in all mammalian cells and are essential for the regulation of normal cell proliferation, differentiation and death (Heby, 1981, Thomas and Thomas, 2001). In every cell there is a pool of polyamines, which is tightly controlled by biosynthesis, catabolism and transport in and out of the cell (Casero and Pegg, 1993). Non-growing cells contain low levels of polyamines and cell proliferation requires an increase in the polyamine pools (Heby, 1981). Polyamine pool depletion is achieved by treating cells with e.g. the spermine analogue N^1,N^{11}-diethylnorspermine (DENSPM, also known as BENSPM or DE-333). DENSPM up-regulates the polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase (SSAT) and inhibits the two major biosynthetic enzymes, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase (AdoMetDC) by feedback inhibition. A high intracellular level of DENSPM activates antizyme, which among other actions also inhibits cellular polyamine uptake. These effects of DENSPM on polyamine metabolism result in a rapid depletion of all polyamines in the cells (Fogel-Petrovic et al., 1997).

In the present study, we investigated the effect of DENSPM-induced polyamine deficiency in MCF-7 and L56Br-C1 breast adenocarcinoma cell lines on the DNA ligase I, FEN1 and PCNA proteins, which are involved in the elongation of the lagging DNA strand. MCF-7 and L56Br-C1 are human breast cancer cell lines with different genetic backgrounds and they react very differently to DENSPM treatment. Initially DENSPM treatment inhibits cell proliferation in both MCF-7 and L56Br-C1 cells, but the more sensitive L56Br-C1 cell line then enters a rapidly progressing apoptotic process (Hegardt et al., 2002, Holst and Oredsson, 2005). We have studied the effect of polyamine deficiency on the expression and localization of DNA ligase I, FEN1 and PCNA, all which are key enzymes in the maturation of Okazaki fragments.

2. Materials and methods

2.1. Materials

Tissue culture plastics were purchased from Nunc, Roskilde, Denmark and cell culture medium components were purchased from Biochrom KG, Berlin, Germany. DENSPM was purchased from Tocris Cookson Ltd., Bristol, UK. Dr. Helmut Mett at Ciba Geigy, Basel, Switzerland, kindly provided 4-amidinoindan-1-one 2′-amidinohydrazone (CGP 48664, also known as SAM 486A). DENSPM and CGP 48664 were made as 2 mM stock solutions in phosphate-buffered saline (PBS, 8 g/l NaCl, 0.2 g/l KCl, 1.15 g/l $NaHPO_4$, 0.2 g/l KH_2PO_4, pH 7.3). The stock solutions were sterilized by filtration, aliquoted and stored at −20 °C. The RNeasy® Midi purification kit was purchased from Qiagen, Valencia, CA, USA. Hybond™ enhanced chemiluminescence (ECL™) nitrocellulose membrane, ECL Advance Western Blotting Detection Kit, GFX purification columns, Cy3-dCTP, Cy5-dCTP and CyScribe GFX Purification kit were purchased from Amersham Biosciences AB, Uppsala, Sweden. The Pronto!™ *Plus* Direct System, Pronto! Universal Hybridization Solution and Pronto! Universal Microarray Hybridization Kit were purchased from Corning Inc., Acton, MA, USA. The Promega Wizard Genomic DNA Purification kit and normal male genomic DNA were purchased from Promega Corporation, Madison, WI, USA. Propidium iodide was purchased from ICN Biomedicals Inc. Irvine, CA, USA. Ribonuclease A type II (RNase A) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Mouse monoclonal DNA ligase I (ab615) and FEN1 (ab462) antibodies were purchased from Abcam, Cambridge, UK. Mouse monoclonal PCNA (clone PC10) antibodies and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG secondary antibodies were purchased from DAKO, Glostrup, Denmark. Alexa Fluor® 488 goat anti-mouse antibodies were purchased from Molecular Probes, Eugene, OR, USA. All components of the NuPAGE® Novex Pre-Cast Gel System used for Western blot were purchased from Invitrogen Corporation, Carlsbad, CA, USA, as well as Trizol reagent. Universal Human Reference RNA was purchased from Stratagene, La Jolla, CA, USA. Polysine™ (poly-L-lysine-coated) glass slides were purchased from Menzel-Gläser®, Braunschweig, Germany.

The MFC-7, SK-BR-3 and HCC1937 cell lines were obtained from the American Type Culture Collection. The