Biochemical and Biophysical Research Communications 442 (2013) 51–55

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Large changes in NAD levels associated with CD38 expression during HL-60 cell differentiation

Zainab N. Al-Abady, Barbara Durante, A. John Moody, Richard A. Billington *

School of Biological Sciences, Plymouth University, Plymouth PL4 8AA, UK

ARTICLE INFO

Article history:
Received 29 October 2013
Available online 9 November 2013

Keywords:
NAD(P)
CD38
HL-60
CLL
CD157
Pyridine nucleotides

ABSTRACT

NAD is an important cofactor involved in multiple metabolic reactions and as a substrate for several NAD-dependent signalling enzymes. One such enzyme is CD38 which, alongside synthesising Ca^{2+}-releasing second messengers and acting as a cell surface receptor, has also been suggested to play a key role in NAD^+ homeostasis. CD38 is well known as a negative prognostic marker in B-CLL but the role of its enzymatic activity has not been studied in depth to date. We have exploited the HL-60 cell line as a model of inducible CD38 expression, to investigate CD38-mediated regulation intracellular NAD^+ levels and the consequences of changes in NAD^+ levels on cell physiology. Intracellular NAD^+ levels fell with increasing CD38 expression and this was reversed with the CD38 inhibitor, kuromanin confirming the key role of CD38 in NAD^+ homeostasis. We also measured the consequences of CD38 expression during the differentiation on a number of functions linked to NAD^+ and we show that some but not all NAD^+-dependent processes are significantly affected by the lowered NAD^+ levels. These data suggest that both functional roles of CD38 might be important in the pathogenesis of B-CLL.

1. Introduction

The pyridine nucleotides nicotinamide adenine dinucleotide (NAD) and its phosphorylated form NADP have long been known to be essential co-enzymes in some of the most fundamental reaction pathways of basic metabolism such as glycolysis, the TCA cycle and the pentose phosphate pathway [1]. It has become clear over the past two decades that their roles in cells extend far beyond being simple electron carriers and NAD(P) has also been shown to be a substrate for enzymes that control pathways of DNA repair (via poly ADP-ribose polymerase; PARP), post-translational protein modification (via ADP-ribosyl transferases; ARTs), gene expression (via sirtuins) and Ca^{2+}-signalling (via CD38/CD157; [2–5]). This has led to a renewed interest in the pathways of NAD(P) homeostasis as it is clear that both the oxidation state and absolute levels of NAD(P) will affect cell physiology via a number of pathways. There has also been much interest in these homeostasis pathways as potential pharmacological targets for a wide variety of diseases.

When NAD(P) is used as a substrate rather than as a redox co-enzyme, the result is that the NAD(P) is consumed with all of the reactions above leading to cleavage of the nicotinamide moiety and generation of free nicotinamide along with compounds containing an ADP-ribose (phosphate) group. In order to maintain NAD(P) levels, three distinct pathways exist to re-synthesise NAD(P) [6]. Of the pathways that consume NAD(P), the most important in terms of the general control of NAD(P) levels would appear to be that mediated by the enzyme CD38 as it is apparently constitutively active [7]. Other pathways such as the PARP pathway may also significantly affect intracellular NAD(P) levels under certain conditions (i.e. DNA damage for PARP) but such changes are likely to be transient.

CD38 is an unusual protein in that it possesses both a receptor function, mediating cell–cell contact and proliferation, and an enzymatic activity [8]. Furthermore, the enzymatic activity is unusual in itself in that the enzyme will use multiple pyridine nucleotide substrates and produce multiple products through at least three known enzymatic mechanisms. A number of the enzymatic products of CD38 have been shown to be involved in cell signalling pathways, for instance, cADPR, NAADP and ADPR [9]. While CD38 is clearly an important regulator of the synthesis of second messengers, recent evidence from the CD38 KO mouse has suggested that the principle role of CD38 may be in the control of NAD(P) levels as the KO mouse showed significantly higher tissue NAD levels than the wild-type [10]. CD38 is perhaps best known for being a prognostic marker for chronic lymphocytic leukaemia (CLL) [8]. Briefly, high levels of CD38 expression correlate with both disease stage and poor prognosis. While the receptor functions of CD38 undoubtedly contribute to high levels of cell proliferation in advanced CLL, the contribution of the enzymatic activity in pathogenesis has remained largely ignored.

* Corresponding author. Fax: +44 01752 584605.
E-mail address: richard.billington@plymouth.ac.uk (R.A. Billington).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.170

Here we have made use of a CD38 expression model, HL-60 cell differentiation by all-*trans* retinoic acid (ATRA), to mimic what might happen to intracellular NAD levels upon the induction of CD38 expression in CLL cells. CD38 expression increased robustly over the first 24 h of differentiation when measured by mRNA, FACS or CD38 activity. Concomitant with this rise in CD38 expression was a significant drop in NAD levels intracellularly that was inhibitable by the CD38 inhibitor, kuromanin [11]. The drop in intracellular NAD levels was accompanied by increased oxidative damage but not by any significant changes in glycolysis. We propose that increased CD38 expression during CLL may have significant effects on NAD levels and that the associated physiological changes may contribute in some way to the pathogenesis of the disease.

2. Materials and methods

2.1. Cell culture

HL-60 and RAJI cells were maintained in suspension in RPMI-1640 medium (Sigma) supplemented with 10% heat-inactivated FCS (Lonza), 100 U/ml penicillin, 100 μg/ml streptomycin (Lonza), and 2 mM L-glutamine (Lonza). Cells were maintained in a humidified incubator supplied with 5% CO_2, at 37 °C. To induce HL-60 differentiation 1 μM all-*trans* retinoic acid (ATRA; Sigma) was added to cells (0.2×10^6 ml^{-1}) [12].

2.2. mRNA isolation and quantitative real-time PCR

Total RNA was isolated using a GenElute kit (Sigma). RNA (1 μg) from control and ATRA-treated HL-60 cells was reversed transcribed to cDNA using MMLV in a GeneAmp PCR System 9700 instrument. RNA was denatured at 70 °C for 10 min in the presence of dNTPs (0.5 mM) and random nonamers (1 μM). Reactions were cooled on ice for 5 min and then 1 unit of MMLV-reverse transcriptase was added to each reaction. Reactions were then incubated at room temperature for 10 min, 37 °C for 50 min and 94 °C for 5 min. cDNA samples were stored at 4 °C until use. Primers (Table 1) were purchased from Eurofins MWG Operon (Germany).

Quantitative RT-PCR was performed using 96 well plates using the StepOne plus sequence detection system (Applied Biosystems, UK). GAPDH was used as a housekeeping gene; cDNA was amplified by adding 2 μl to a final reaction volume of 25 μl containing SYBR green, 0.05 U/μl Taq polymerase, 300 nM reference dye and 0.2 μM of the specific primers. Reactions were performed under the following conditions: 94 °C for 2 min followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. The *Ct* value from control was compared with that of ATRA-treated cells using the $\Delta\Delta CT$ methodology to determine the relative target quantity.

Table 1
Primers used for qPCR experiments.

Genes	Primers	Size	Product (bp)
GAPDH	For:CCCACTCCTCCACCTTTGAC Rev:CTGTTGCTGTAGCCAAATTCGT	20 22	100
CD38	For: GCACCACCAAGCGCTTTC Rev:TCCCATACACTTTGGCAGTCTACA	18 24	100
CD157	For: GGGAAGGCAGCATGAAAGTC Rev: GGTCCACGCACTGTAAGAGCTT	20 22	105
IDO	For: GCCTGCGGGAAGCTTATG Rev: TGGCTTGCAGGAATCAGGAT	18 20	100
NMNAT	For: TCATTCAATCCCATCACCAACA Rev: AGGAGAGATGATGCCTTTGACAA	22 23	105
NAMPT	For: TCCGGCCCGAGATGAAT Rev: TGCTTGTGTTGGGTGGATATTG	17 22	105

2.3. Measurement of ADP-ribosyl cyclase activity

The cyclase activity was assayed by monitoring the conversion of nicotinamide guanine dinucleotide (NGD) into cyclic GDP-ribose (cGDPR), as described earlier [13]. The reaction was started by adding 2.0×10^6 cells to a reaction mixture containing 100 μM NGD in PBS, pH 7.4. The production of the fluorescent cGDPR was monitored for 5–10 min, measured fluorimetrically (excitation 300 nm; emission 410 nm) with a LS50B fluorimeter (Perkin Elmer, UK).

2.4. FACS analysis

Cells were resuspended in 100 μl of FACS staining buffer (PBS containing 1% BSA) and incubated for 30 min at 4 °C, followed by three washes. Cells (15 μl) were incubated with either the conjugated monoclonal antibody anti-CD38 (1:20; eBiosciences, UK), or the isotype control-PE (1:20) for 30 min at 4 °C in the dark. After incubation cells were washed twice with 500 μl of FACS buffer and resuspended in 500 μl PBS. The samples were sorted on an Arial II FACS (Becton–Dickinson, USA), and data from 10,000 events were collected and analysed using FACS Diva version 6.1.3 software.

2.5. NAD^+/NADH determination

Intracellular NAD^+ and NADH levels were determined by enzymatic cycling assay ostensibly as previously described [14]. Cells ($1.25–5 \times 10^6$ ml^{-1}) were pelleted and frozen in a dry ice-ethanol bath. For NAD^+ extraction, 250 μl of 0.2 M HCl was added to the frozen pellets, while 250 μl of 0.2 M NaOH was used for NADH extraction. Samples were placed in a 100 °C water bath for 10 min and then centrifuged at 5000*g* for 5 min. The supernatants were stored in −20 °C until needed. Extracted NAD(H) (49 μl) was added to 151 μl of a reaction mixture containing 98 mM Bicine (pH 8.0), 24 mM of either NaOH (for NAD^+ extraction) or HCl (for NADH extraction), 1.62 mM PES, 0.41 mM MTT, 19.6 μl ethanol, 3.92 mM EDTA and 5 μl of yeast ADH (400 U ml^{-1}). The absorbance at 565 nm was recorded in a plate reader (VersaMax, Molecular Devices, Sunny vale, CA) after 30 min incubation in the dark. NAD^+ and NADH standards (5–60 μM) were used for calibration.

2.6. Lactate assay

Medium (50 μl) or lactate standard solution (0.1–1 mM) was mixed with 250 μl of assay mixture containing 315.8 mM glycine, 252.6 mM hydrazine, 4 mM NAD^+ and 16.6 U ml^{-1} lactate dehydrogenase (LDH). The reaction was incubated at 37 °C for 30 min and measured at 340 nm in a plate reader. Data were normalised to cell number.

2.7. Thiobarbituric acid reactive substance assay

Cell pellets ($5–10 \times 10^6$ cells) were re-suspended in 200 μl ice-cold PBS and sonicated for 15 s on ice. Ice-cold 10% TCA (400 μl) was added and the reaction was centrifuged at 15,000*g* for 5 min at 4 °C. 4.6 mM thiobarbituric acid (200 μl) was added to an equal volume of each supernatant. The colour was developed at 100 °C for 30 min and the reaction was stopped by cooling for 5 min. Each sample (300 μl) was transferred to a 96-well plate and the absorbance at 532 nm was recorded with reference to a reagent blank. The assay was calibrated with 1,1,3,3-tetraethoxypropane (0–100 μM).

2.8. Statistical analysis

Statistical analysis of the data was performed with the Stat View statistical software (Abacus concepts, California, USA) using the one way ANOVA analysis.

3. Results and discussion

CD38 has been suggested to be a major regulator of NAD(P) levels in mammalian cells and is also known as a negative prognostic marker in CLL. In order to mimic the changes in expression of CD38 during disease progression in CLL, we have made use of the well-characterised HL-60 differentiation model. HL-60 cells were stimulated to differentiate by addition of 1 μM ATRA and cells were left to differentiate into neutrophil-like cells for up to 5 days [12]. By the end of the treatment, the cells had acquired the ability to reduce NBT and thus were deemed to be differentiated (data not shown; [15]). We measured CD38 mRNA expression via qPCR, protein expression via FACS and we also measured CD38 enzymatic activity using the NGD assay. CD38 mRNA levels increased rapidly and robustly over the first 24 h of differentiation, reaching levels approximately 500-fold higher than in undifferentiated cells, and then stayed high throughout the remaining 4 days (Fig. 1A). Expression was first observed from 3 h. We also measured the expression of the CD38 homologue, CD157 but only a slight increase was observed (6.70 ± 0.15-fold increase over 5 days; Fig. 1A). CD38 enzymatic activity appeared on the plasma membrane, as measured using the conversion of NGD to cGDPR in intact cells, over a similar time scale, with a peak in activity at 6 h that then levelled off (Fig. 1B). It is unclear as to why the activity peaked and then dropped to a lower level but one possibility is that the CD38 is active immediately after transcription and on arrival at the plasma membrane, and that it is then redistributed within the cell via recycling pathways. This data confirms that active CD38 is present soon after the initiation of differentiation. Protein expression of CD38 was confirmed via FACS analysis and showed large increases in protein levels already by 10 h of differentiation (Fig. 1C).

In order to measure whether CD38 expression had any effect on intracellular NAD^+ levels, we extracted intracellular NAD^+ at different time points after the initiation of differentiation (Fig. 2A). NAD^+ levels decreased rapidly within the first day of differentiation and continued to decrease more slowly between 1 and 5 days of treatment. NAD^+ levels were 64.6 ± 8.9% of control after 24 h and 40.5 ± 8.7% of control after 5 days. Previous work has shown that CD38 is a major regulator of intracellular NAD^+ levels and our results support that hypothesis [7]. In order to test whether the correlation between the NAD^+ levels and CD38 activity/expression was likely to be due to CD38, we made use of a recently discovered CD38 inhibitor, the flavonoid, kuromanin [11]. Luteolin was also found to inhibit CD38 but this compound has previously been shown to inhibit glycolysis [16] and thus, we thought it best to avoid its use. While we cannot be sure of any non-CD38 mediated effects of kuromanin, there was no effect of prolonged kuromanin treatment on cell vitality (data not shown). Nevertheless, the use of kuromaninwas limited to a single, short time point (6 h) to best avoid any potential confounding effects. Kuromanin (10 μM) effectively reversed the drop in NAD^+ levels suggesting that this effect is CD38 dependent (Fig. 2B). Additionally, we found no such reversal in the presence of 4-amino-1,8-naphthalimide or sirtinol, inhibitors of PARP and Sirt1/2, respectively [17,18], suggesting that the other major NAD^+-consuming pathways are not involved (data not shown).

Much has been made of the observation that CD38 is an ectoenzyme, and thus, its active site is not intracellular and in contact

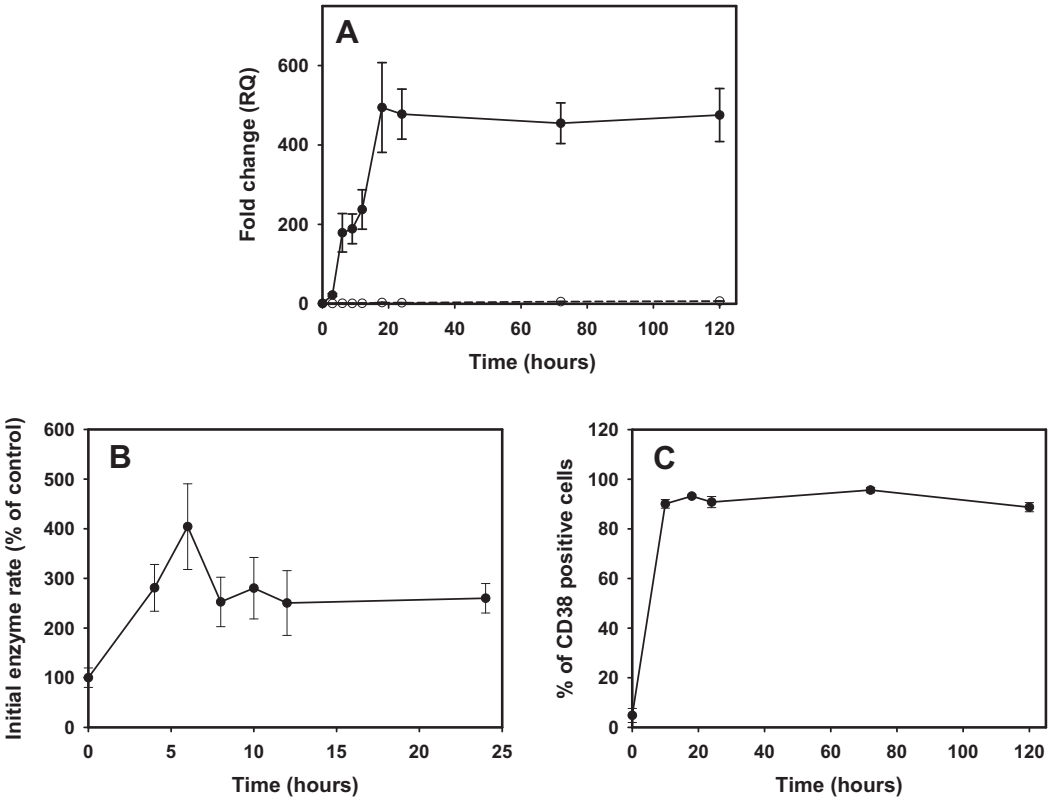

Fig. 1. CD38 expression during differentiation. (A) mRNA expression of CD38 (solid line) and CD157 (dashed line), $n = 3$ (B) plasma membrane CD38 cyclase activity $n = 3$ (C) FACS analysis of extracellular CD38 expression. Data are expressed as mean ± SEM of 3 separate cultures.

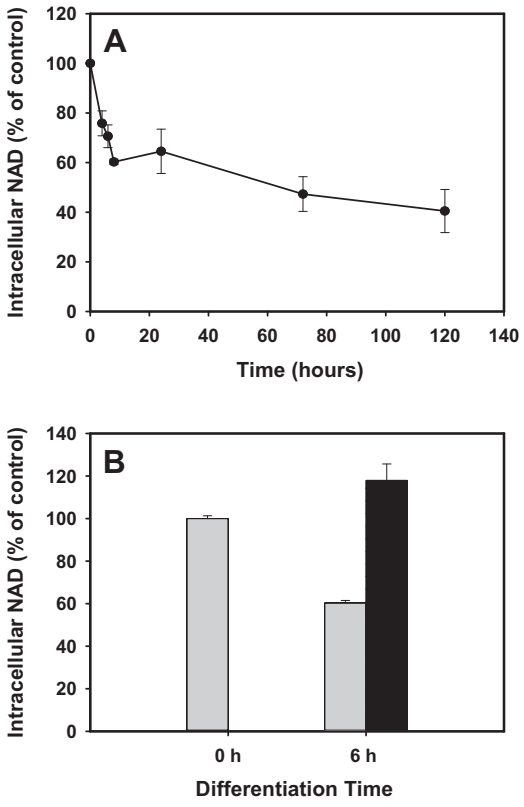

Fig. 2. (A) The decline in intracellular NAD^+ levels during HL-60 differentiation, $n = 3$ (B) The effect of treatment with 10 μM kuromanin on NAD^+ levels. Data are expressed as mean ± SEM of three separate cultures.

with the NAD^+. This topological paradox has caused some controversy but recent work has clarified this problem and it appears that CD38 can flip-flop across the plasma membrane (and one would assume, organelle membranes), and thus its active site can be in the same compartment as the cytosolic NAD^+ [19]. These data have profound consequences for cells with inducible CD38 expression as induction of expression may lead to significant drops in intracellular NAD levels which may impact on both basic metabolism and on cell signalling pathways including both gene expression regulation and DNA repair pathways. This might be particularly true for cancer cells as they tend to rely on anaerobic glycolysis as their major source of ATP. Preferentially using this NAD-dependent pathway even in the presence of adequate oxygen levels [20]. For CLL, disease progression is marked by changes in CD38 expression which correlate with poor prognosis and a more aggressive phenotype [2]. We hypothesise that the initiation of CD38 expression in CLL will be accompanied by similar effects on NAD^+ levels. Interestingly, despite an upregulation of an NAD^+ degrading pathway, we saw no significant increase in the expression of the key enzymes of the NAD-recycling pathway, an increase in which might act to counteract any drop in NAD^+ levels (Table 2). The only significant change that we observed was a large increase in the expression of the *de novo* NAD synthesis pathway enzyme IDO (indoleamine 2,3 dioxygenase) after 5 days of differentiation. However, as the product of this pathway is the NAD precursor nicotinic acid (later converted to nicotinamide by NAD synthetase), this is unlikely to have a relevant effect on NAD levels as niacin is present in the medium and as a breakdown product of CD38 meaning that it is not a limiting factor [21]. The expression of IDO more likely reflects the differentiation to a new cell type [22].

In order to test the effect of the drop in NAD^+ levels on NAD-dependent processes in control and differentiating cells, we measured NAD^+/NADH ratio, glycolytic flux (via lactate accumulation) and accumulated lipid peroxidation using the TBARS assay (Fig. 3). Strikingly, we found that NAD^+ and NADH levels both dropped similarly such that no large differences in NAD^+/NADH ratio were observed between control and differentiating cells (Fig. 3A). This might seem unusual as one would expect that the ratio would change due to NADH production via glycolysis. However, we found no reduction in accumulated lactate (nmol/10^6 cells) suggesting that glycolytic activity was largely unaffected (data not shown) and that would fit well with the NAD^+/NADH data as lowered NAD^+ and a high level of glycolysis would be expected to shift the ratio towards NADH. It is worth mentioning that ATRA-differentiated HL-60 cells retain only vestigial mitochondria and thus rely on anaerobic glycolysis for ATP production [23]. We did find a significant difference between control and differentiating cells with respect to accumulation of products of lipid peroxidation, suggesting a reduction in antioxidant defence (Fig. 3C). These data suggest that the drop in NAD levels associated with CD38 expression has potentially significant consequences for the physiology of cells beginning to express CD38. It is perhaps hard to rationalise the unaltered glycolytic activity with the phenotype of CD38 positive CLL cells that display increased proliferative activity. However, the drop in NAD levels is not as catastrophic as that caused by agents such as the NAD recycling inhibitor, FK866, which lowers NAD to undetectable levels in short periods of time [24]. Indeed, our data suggest that glycolysis is still active and that the NAD pool is still large enough to maintain the NAD^+/NADH ratio. How this drop in NAD(H) levels might affect ATP generation capacity under periods of stress or high levels of proliferation stimuli may be worthwhile pursuing in $CD38^+$ CLL cells.

Our data provide further evidence for the hypothesis that CD38 is a major and physiologically relevant regulator of NAD levels in

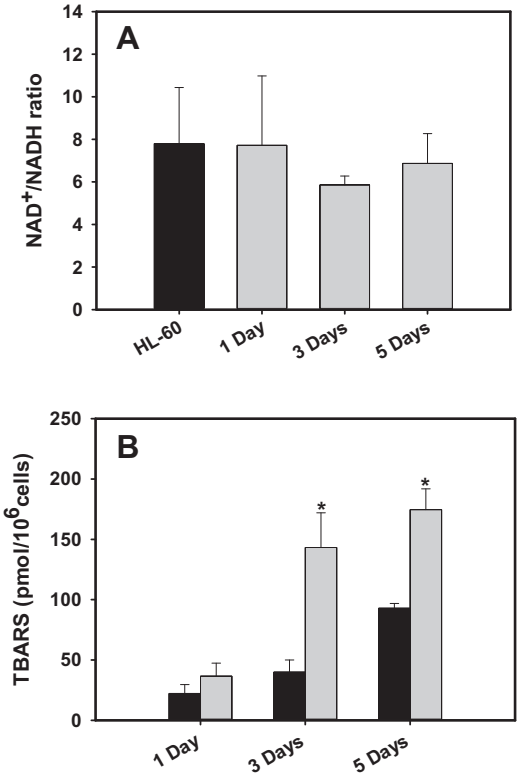

Fig. 3. Consequences of NAD^+ depletion on (A) NAD^+/NADH ratio and (B), TBARS production during HL-60 differentiation. Data are expressed as mean ± SEM of three separate cultures. Differences between groups were assessed by one-way ANOVA. $^*P < 0.05$ versus control.

Table 2
Expression of NAD-homeostasis enzymes during ATRA-induced differentiation of HL-60 cells, data are expressed as mean ± SEM of 3 separate cultures.

Time (h)	IDO	NMNAT	NAMPT
0	1.00 ± 0.03	1.00 ± 0.03	1.00 ± 0.03
24	1.63 ± 0.56	1.00 ± 0.09	1.31 ± 0.41
72	9.52 ± 0.45	1.35 ± 0.11	1.41 ± 0.29
120	106.87 ± 32.55	2.09 ± 0.23	1.78 ± 0.46

mammalian cells [10]. Given the striking changes in phenotype in CLL cells during disease progression as they begin to express CD38, we propose that, together with the well-known effects of the receptorial function of CD38, the effects of the enzymatic function need further study as this may act in concert with the receptorial function [8]. It is likely that the changes that we observe in NAD levels will have consequences for cell physiology on a number of signalling processes that may include DNA repair mechanisms and gene expression. In particular, lowered NAD levels may lead to reduced activity of the PARP-mediated DNA repair pathway with an associated increase in the potential for genomic instability that, when coupled with increased proliferation rates, might play a role in disease progression [25]. Furthermore, the reaction performed by CD38 on NAD will produce nicotinamide and ADPR (perhaps via cADPR). Nicotinamide is well known as an inhibitor of many of the enzymes that consume NAD including CD38 itself, PARPs and sirtuins [26–28]. It is perhaps interesting to note that HL-60 cells, during differentiation by ATRA have been shown to have low PARP activity and this may be due to lowered substrate concentration or increased inhibitor concentration with NAD degradation [29]. ADPR has also been shown to be a signalling molecule in its own right [30]. Lowered NAD levels will also impinge in the long term on substrate availability for CD38 itself in cADPR synthesis reactions which might go some way to explaining why, despite increased CD38 expression, cADPR signalling has not been found to correlate with this expression in CLL cells [31]. Indeed, the data presented here raise an important paradox between CD38 expression being required for the generation of cADPR and potentially lowered levels of the substrate in all cell systems. This paradox may also apply to enzymatic synthesis of NAADP.

Acknowledgment

The authors thank the Iraqi Ministry of Higher Education and Scientific Research (MOHESR) for a PhD studentship to ZA.

References

[1] M. Ziegler, New functions of a long-known molecule. Emerging roles of NAD in cellular signaling, Eur. J. Biochem. 267 (2000) 1550–1564.
[2] F. Malavasi, S. Deaglio, A. Funaro, E. Ferrero, A.L. Horenstein, E. Ortolan, T. Vaisitti, S. Aydin, Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology, Physiol. Rev. 88 (2008) 841–886.
[3] M.Y. Kim, T. Zhang, W.L. Kraus, Poly(ADP-ribosyl)ation by PARP-1: 'PAR-laying' NAD^+ into a nuclear signal, Genes Dev. 19 (2005) 1951–1967.
[4] S. Michan, D. Sinclair, Sirtuins in mammals: insights into their biological function, Biochem. J. 404 (2007) 1–13.
[5] F. Koch-Nolte, S. Kernstock, C. Mueller-Dieckmann, M.S. Weiss, F. Haag, Mammalian ADP-ribosyltransferases and ADP-ribosylhydrolases, Front Biosci. 13 (2008) 6716–6729.
[6] G. Magni, A. Amici, M. Emanuelli, G. Orsomando, N. Raffaelli, S. Ruggieri, Enzymology of NAD^+ homeostasis in man, Cell. Mol. Life Sci. 61 (2004) 19–34.
[7] F. Malavasi, S. Deaglio, G. Zaccarello, A.L. Horenstein, A. Chillemi, V. Audrito, S. Serra, M. Gandione, A. Zitella, A. Tizzani, The hidden life of NAD^+-consuming ectoenzymes in the endocrine system, J. Mol. Endocrinol. 45 (2010) 183–191.
[8] A. Funaro, F. Malavasi, Human CD38, a surface receptor, an enzyme, an adhesion molecule and not a simple marker, J. Biol. Regul. Homeost. Agents 13 (1999) 54–61.
[9] H.C. Lee, Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization, J. Biol. Chem. 287 (2012) 31633–31640.
[10] M.T. Barbosa, S.M. Soares, C.M. Novak, D. Sinclair, J.A. Levine, P. Aksoy, E.N. Chini, The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity, FASEB J. 21 (2007) 3629–3639.
[11] E. Kellenberger, I. Kuhn, F. Schuber, H. Muller-Steffner, Flavonoids as inhibitors of human CD38, Bioorg. Med. Chem. Lett. 21 (2011) 3939–3942.
[12] T.R. Breitman, S.E. Selonick, S.J. Collins, Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid, Proc. Natl. Acad. Sci. USA 77 (1980) 2936–2940.
[13] A.J. Morgan, G.C. Churchill, R. Masgrau, M. Ruas, L.C. Davis, R.A. Billington, S. Patel, M. Yamasaki, J.M. Thomas, A.A. Genazzani, A. Galione, Methods in cyclic ADP-ribose and NAADP research, second ed., Calcium Signaling, CRC Press, 2005, pp. 265–334.
[14] M.R. Leonardo, Y. Dailly, D.P. Clark, Role of NAD in regulating the adhE gene of *Escherichia coli*, J. Bacteriol. 178 (1996) 6013–6018.
[15] R.L. Baehner, L.A. Boxer, J. Davis, The biochemical basis of nitroblue tetrazolium reduction in normal human and chronic granulomatous disease polymorphonuclear leukocytes, Blood 48 (1976) 309–313.
[16] G.J. Du, Z.H. Song, H.H. Lin, X.F. Han, S. Zhang, Y.M. Yang, Luteolin as a glycolysis inhibitor offers superior efficacy and lesser toxicity of doxorubicin in breast cancer cells, Biochem. Biophys. Res. Commun. 372 (2008) 497–502.
[17] D.C. Hegan, Y. Lu, G.C. Stachelek, M.E. Crosby, R.S. Bindra, P.M. Glazer, Inhibition of poly(ADP-ribose) polymerase down-regulates BRCA1 and RAD51 in a pathway mediated by E2F4 and p130, Proc. Natl. Acad. Sci. USA 107 (2010) 2201–2206.
[18] C.A. Fernandes, L. Fievez, A.M. Neyrinck, N.M. Delzenne, F. Bureau, R. Vanbever, Sirtuin inhibition attenuates the production of inflammatory cytokines in lipopolysaccharide-stimulated macrophages, Biochem. Biophys. Res. Commun. 420 (2012) 857–861.
[19] Y.J. Zhao, C.M. Lam, H.C. Lee, The membrane-bound enzyme CD38 exists in two opposing orientations, Sci. Signal. 5 (2012) ra67.
[20] O. Warburg, On the origin of cancer cells, Science 123 (1956) 309–314.
[21] D.A. Bender, R. Olufunwa, Utilization of tryptophan, nicotinamide and nicotinic acid as precursors for nicotinamide nucleotide synthesis in isolated rat liver cells, Br. J. Nutr. 59 (1988) 279–287.
[22] K. Van der Sluijs, R. Singh, A. Dijkhuis, M. Snoek, R. Lutter, Indoleamine-2,3-dioxygenase activity induces neutrophil apoptosis, Crit. Care 15 (2011) P208.
[23] B.J. van Raam, W. Sluiter, E. de Wit, D. Roos, A.J. Verhoeven, T.W. Kuijpers, Mitochondrial membrane potential in human neutrophils is maintained by complex III activity in the absence of supercomplex organisation, PLoS ONE 3 (2008) e2013.
[24] R.A. Billington, A.A. Genazzani, C. Travelli, F. Condorelli, NAD depletion by FK866 induces autophagy, Autophagy 4 (2008) 385–387.
[25] S.M. Henning, M.E. Swendseid, W.F. Coulson, Male rats fed methyl- and folate-deficient diets with or without niacin develop hepatic carcinomas associated with decreased tissue NAD concentrations and altered poly(ADP-ribose) polymerase activity, J. Nutr. 127 (1997) 30–36.
[26] C.B. Munshi, R. Graeff, H.C. Lee, Evidence for a causal role of CD38 expression in granulocytic differentiation of human HL-60 cells, J. Biol. Chem. 277 (2002) 49453–49458.
[27] S.D. Park, C.G. Kim, M.G. Kim, Inhibitors of poly(ADP-ribose) polymerase enhance DNA strand breaks, excision repair, and sister chromatid exchanges induced by alkylating agents, Environ. Mutagen. 5 (1983) 515–525.
[28] V. Audrito, T. Vaisitti, D. Rossi, D. Gottardi, G. D'Arena, L. Laurenti, G. Gaidano, F. Malavasi, S. Deaglio, Nicotinamide blocks proliferation and induces apoptosis of chronic lymphocytic leukemia cells through activation of the p53/miR-34a/SIRT1 tumor suppressor network, Cancer Res. 71 (2011) 4473–4483.
[29] M. Kanai, M. Miwa, T. Kondo, Y. Tanaka, M. Nakayasu, T. Sugimura, Involvement of poly (ADP-ribose) metabolism in induction of differentiation of HL-60 promyelocytic leukemia cells, Biochem. Biophys. Res. Commun. 105 (1982) 404–411.
[30] A.L. Perraud, A. Fleig, C.A. Dunn, L.A. Bagley, P. Launay, C. Schmitz, A.J. Stokes, Q. Zhu, M.J. Bessman, R. Penner, J.P. Kinet, A.M. Scharenberg, ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by nudix motif homology, Nature 411 (2001) 595–599.
[31] S. Deaglio, T. Vaisitti, R. Billington, L. Bergui, P. Omede, A.A. Genazzani, F. Malavasi, CD38/CD19: a lipid raft-dependent signaling complex in human B cells, Blood 109 (2007) 5390–5398.