Biochemical and Biophysical Research Communications 473 (2016) 789–794

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Imiquimod induces ER stress and Ca^{2+} influx independently of TLR7 and TLR8

William A. Nyberg, Alexander Espinosa*

Unit of Experimental Rheumatology, Center for Molecular Medicine, L8:04, Karolinska University Hospital, Karolinska Institutet, Sjukhusringen 6, SE-171 76, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 29 February 2016
Accepted 18 March 2016
Available online 19 March 2016

Keywords:
Endoplasmic-reticulum stress
Imidazoquinolines
Imiquimod
Toll-like receptor
Ca^{2+} influx

ABSTRACT

Endoplasmic reticulum (ER) stress is a physiological response to protein overload or misfolded proteins in the ER. Certain anti-cancer drugs, e.g. bortezomib and nelfinavir, induce ER stress implying that this could be a successful therapeutic strategy against several forms of cancer. To find novel ER-stress inducers we screened a panel of natural and synthetic Toll-like receptor (TLR) agonists against human keratinocytes and identified the anti-cancer drug imiquimod (IMQ) as a potent inducer of ER stress. Other TLR7 and TLR8 agonists, including resiquimod and gardiquimod, did not induce ER stress, demonstrating that IMQ induces ER stress independently of TLR7 and TLR8. We further confirmed this by showing that IMQ could still induce ER stress in mouse *Tlr7*$^{-/-}$ cells. IMQ also induced a rapid and transient influx of extracellular Ca^{2+} together with the release of Ca^{2+} from internal stores. Depletion of Ca^{2+} from the ER is a known cause of ER stress suggesting that IMQ induces ER stress via depletion of ER Ca^{2+}. The ER-stress inducing property of IMQ is possibly of importance for its efficacy in treating basal cell carcinoma, in situ melanoma, and squamous cell carcinoma. Our data could potentially be harnessed for rational design of even more potent ER-stress inducers and new anti-cancer drugs.

1. Introduction

Endoplasmic reticulum (ER) stress is a physiological response that occurs after protein overload, or accumulation of misfolded proteins, in the ER lumen. ER stress triggers the highly conserved unfolded-protein response (UPR) mediated by IRE1-dependent splicing of XBP1 mRNA, proteolytic processing of ATF6 and the phosphorylation of PERK [1,2]. This in turn is followed by the induction of ER-stress target genes including pro- or anti-apoptotic genes, chaperone genes and phospholipid-synthesis genes, e.g. *DDIT3* (CHOP), *ERDJ4*, *GRP78* (BiP), and *EPT1* [3]. Chronic and high levels of ER stress induce cell death whereas moderate or transient ER stress serves a protective role for stressed cells, e.g. during tumor growth [4]. Tipping the balance of ER stress towards cell death has recently emerged as a successful anti-cancer strategy. The anti-cancer drugs bortezomib, nelfinavir and tanespimycin all act via the induction of ER stress followed by apoptosis or reduced growth of cancer cells [5–8]. Since pharmacologically induced ER stress seems effective against certain forms of cancer, a search for additional potent ER-stress inducers is motivated.

Toll-like receptor (TLR) signaling can trigger the activation of XBP1, PERK and ATF6 in macrophages, but with little or no induction of classical ER-stress target genes [9]. In addition, TLR signaling also leads to splicing of XBP1 followed by induction of pro-inflammatory genes in synoviocytes and by apoptosis in intestinal stem cells [10,11]. Since skin responds with ER stress after exposure to several environmental factors, we hypothesized that keratinocytes, in contrast to macrophages, would be prone to a full ER-stress response after exposure to natural or synthetic TLR agonists [12–14]. Indeed, while screening TLR agonists against primary human keratinocytes we identified the synthetic TLR7-agonist imiquimod (IMQ) as a potent inducer of ER stress. IMQ is a purine analog with anti-cancer properties and is the prototypic compound of the imidazoquinolines, a group of small immunostimulatory molecules including resiquimod (RSQ) and gardiquimod (GDQ) [15]. IMQ triggers TLR7 signaling followed by the expression of pro-inflammatory factors such as IFNα, IL6 and TNF [16]. However, it is well established that IMQ also provokes TLR7-independent responses in skin. Topical IMQ cream (Aldara) induces strong responses in skin of *Tlr7*$^{-/-}$ and *Myd88*$^{-/-}$ mice independently of

* Corresponding author. Unit of Experimental Rheumatology, Department of Medicine, Center for Molecular Medicine, CMM L8:04, Karolinska Institutet, SE-171 76, Stockholm, Sweden.
E-mail address: alexander.espinosa@ki.se (A. Espinosa).

http://dx.doi.org/10.1016/j.bbrc.2016.03.080

TLR7 and adaptive immune responses [17,18]. We discovered that IMQ induces ER stress in a TLR7-independent manner in keratinocytes and melanoma cells. Interestingly, other TLR7 and TLR8 agonists, including RSQ and GDQ, were unable to induce ER stress indicating that small structural changes in imidazoquinolines impacts their ER-stress inducing activity.

While trying to understand the mechanism whereby IMQ induces ER stress we discovered that IMQ induced a rapid and transient influx of extracellular Ca^{2+} and a release of intracellular Ca^{2+} stores. Depletion of Ca^{2+} from the ER lumen is a known cause of ER stress, mediated by the interference with Ca^{2+}-dependent chaperones. This suggests that IMQ induces ER stress via depletion of Ca^{2+} from the ER [19]. Identifying the receptor for IMQ that mediates the induction of ER stress could potentially lead to the discovery of endogenous ligands that control physiological ER-stress responses. Furthermore, the data provided here might be useful for rational design of strong ER-stress inducers and new anti-cancer drugs.

2. Material and methods

2.1. Cells

Neonatal human keratinocytes (Life Technologies) were cultured in keratinocyte-SFM medium (Gibco) supplemented with 0.2 ng/ml recombinant epidermal growth factor (EGF) and 30 μg/ml of bovine pituitary extract (BPE) (Gibco). The medium was additionally supplemented with 0.3 mM $CaCl_2$, penicillin (100 U/ml) and streptomycin (100 μg/ml) (Gibco). Human melanoma cell lines A375, SK-MEL-28 and MeWo (ATCC) were cultured in DMEM medium supplemented with 5% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and glutamine (1 mM). Mouse splenocytes were isolated from WT and *Tlr7*$^{-/-}$ mice and were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (1 mM) and β-mercaptoethanol (50 μM).

2.2. Quantitative RT-PCR

Total RNA was isolated using TRIzol (Life Technologies) using chloroform and isopropanol precipitation. After one wash in 70% ethanol the concentration of total RNA was determined using NanoDrop 1000 (Thermo Scientific). Total RNA was reverse transcribed in cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) or iScript cDNA synthesis kit (Bio-Rad). Gene expression levels were determined by real-time PCR using SYBR reagent on an Mx3005P (Agilent Technologies) or TaqMan probes (Thermo Scientific) on a LightCycler 96 (Roche). Primer sequences and more information on SYBR and TaqMan assays are available on request.

2.3. Calcium measurements

A375 were loaded with Fluo-3 AM (1 μM) (Thermo Scientific) in PBS (0.9% Ca^{2+}) buffer containing pluronic acid (0.02%) and

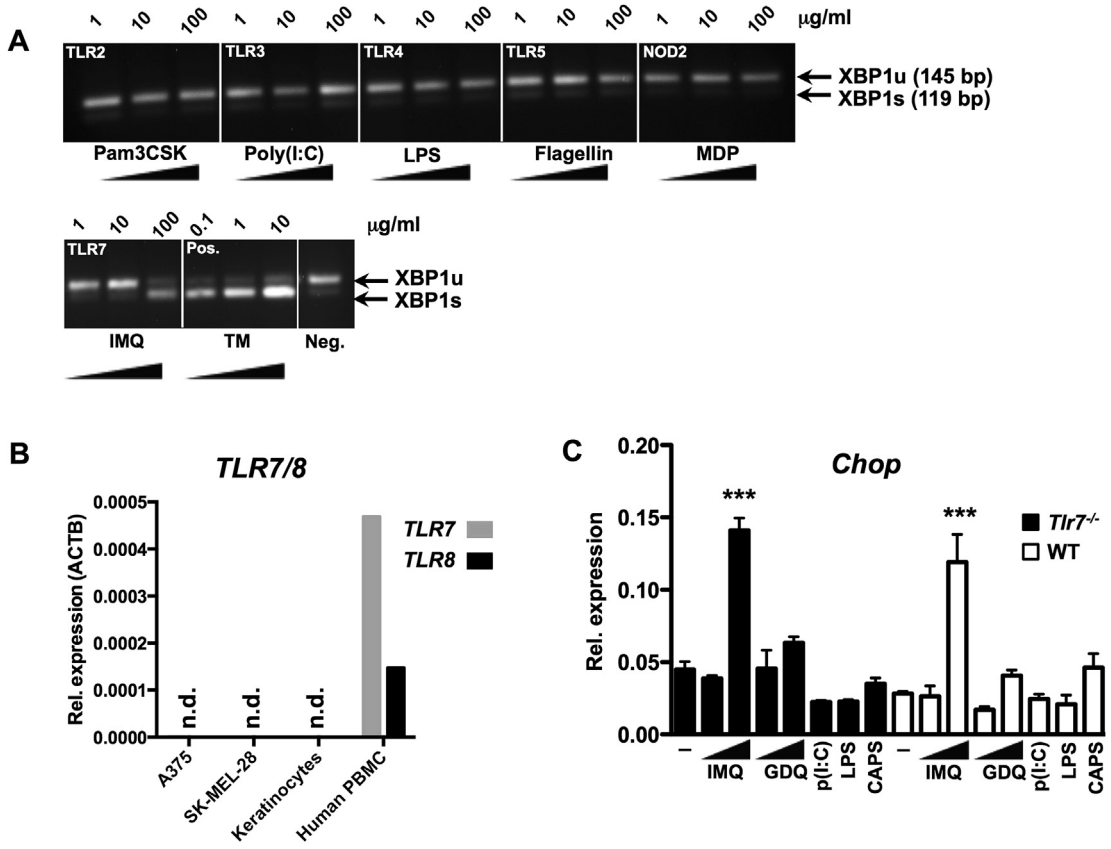

Fig. 1. IMQ induces a TLR7-independent ER-stress response. Using primary human keratinocytes we screened a panel of TLR agonists to identify novel inducers of ER stress. We used XBP1 splicing as a marker for ER stress and identified IMQ as a potent inducer of ER stress in human keratinocytes (A). Primary human keratinocytes and human melanoma cells do not express TLR7 or TLR8, clearly indicating that the IMQ-induced ER-stress response is independent of TLR7 and TLR8 (B). To further verify that the IMQ-induced ER-stress response occurs independently of TLR7 we stimulated mouse *Tlr7*$^{-/-}$ and WT splenocytes with IMQ (C). Indeed, IMQ induced an ER-stress response both in *Tlr7*$^{-/-}$ and WT cells as measured by induction of the ER-stress gene CHOP (Ddit3). The potent TLR7-agonist GDQ could not induce ER stress in neither *Tlr7*$^{-/-}$ nor WT cells. (One-way ANOVA followed by Dunnett's post-test, *** = $p < 0.01$.)

probenecid (1 mM) (Sigma Aldrich). After incubation for 30 min at 25 °C (protected from light) the cells were washed twice in PBS (with 1 mM probenecid) and incubated for another 30 min at 25 °C (protected from light). After a last wash in PBS the cells were acquired. To trigger Ca^{2+} flux cells were treated with imiquimod, CL075, resiquimod (all from Invivogen) at 25 ug/ml. Ionomycin (1 μM) was used as a positive control (Sigma Aldrich). To block extracellular Ca^{2+} we added EGTA (1 mM) before acquisition (Sigma Aldrich). To inhibit CRAC channels we added the CRAC inhibitor BPT2 (10 μM) before acquisition (Calbiochem). All cells were acquired using an LSRII cytometer (Becton Dickinson) using the FACSDiva (Becton Dickinson). Data was analyzed using FlowJo (FlowJo, LLC).

2.4. XBP1 splicing assay

After RNA isolation and reverse-transcription into cDNA, endpoint PCR was performed using the following primers: XBP1-F: CCTGGTTGCTGAAGAGGAGG, and XBP1-R: CCATGGGGA-GATGTTCTGGAG. The PCR program was initiated by a denaturation step at 94 °C for 3 min, followed by 30 cycles of: 30 s 94 °C, 30 s 58 °C, 30 s 72 °C. The program was ended with a 3 min step at 72 °C. The PCR products were separated on a 2.5% agarose gel. Unspliced XBP1 appeared as a 145 bp band, while spliced XBP1 appeared as a 119 bp band.

2.5. Flow cytometry

A375 cells were incubated with IMQ (25 μg/ml) or RSQ (25 μg/ml) in PBS for 15 min at 25 °C followed by two washes with PBS. IMQ was excited with an ultraviolet laser (355 nm) and the fluorescence was detected using the 440/40 filter set. All cells were acquired using an LSRII cytometer (Becton Dickinson) using the FACSDiva (Becton Dickinson). Data was analyzed using FlowJo (FlowJo, LLC).

3. Results

3.1. IMQ induces a strong ER-stress response in primary human keratinocytes independently of TLR7 and TLR8

We screened a panel of natural and synthetic TLR and NOD agonists (TLR2, TLR3, TLR4, TLR5, TLR7 and NOD2) to identify those capable of inducing ER stress in primary human keratinocytes. We used the known ER-stress inducer tunicamycin (TM) as a positive control for XBP1 splicing. After incubation for 6 h we identified ER-stress inducing TLR agonists through an XBP1-splicing assay (Fig. 1A). The only TLR agonist that could induce XBP1 splicing in keratinocytes was IMQ, detected by the appearance of spliced XBP1 (XBP1s) at 119 bp after RT-PCR. The induction of XBP1 splicing by IMQ was concentration dependent and at concentrations ≥25 μg/ml the splicing of XBP1 was near complete in keratinocytes (Supplementary Fig. 1A). IMQ is a well-known TLR7 agonist, however, we did not detect neither TLR7 nor TLR8 expression in human keratinocytes and melanoma cells demonstrating that the ER-stress inducing properties of IMQ are independent of TLR7 and TLR8 (Fig. 1B, Supplementary Fig. 1B). Indeed, several reports have previously determined that human keratinocytes do not express TLR7 and TLR8 [20,21]. We further verified that IMQ acts in a TLR7-independent manner by successfully inducing ER stress both in *Tlr7*$^{-/-}$ and WT mouse splenocytes with IMQ (Fig. 1C). The potent TLR7-agonist GDQ could not induce ER stress neither in *Tlr7*$^{-/-}$ nor WT cells, confirming that IMQ induces ER stress independently of TLR7. In addition, the TRPV1-agonist capsaicin (CAPS) did not induce ER stress demonstrating that IMQ does not act via the receptor TRPV1 as previously suggested [18]. To verify that the IMQ-induced splicing of XBP1 occurred via IRE1 we used S2R+ cells for efficient knockdown of IRE1 followed by stimulation with IMQ for 6 h. In the absence of IRE1, IMQ was unable to induce splicing of XBP1 whereas the control knockdown of GFP did not affect the splicing of XBP1 (Supplementary Fig. 1C). To confirm that IMQ triggers a bona fide ER-stress response we verified that IMQ induced the expression of ER-stress genes including *ERDJ4*, *DDIT3* (CHOP) and *GRP78* (BiP) (Supplementary Fig. 1D).

3.2. Structure–activity relationship among imidazoquinolines

IMQ is the original member of the antiviral imidazoquinolines, a group of purine analogs that are potent TLR7 or TLR8 agonists. Originally developed as antiviral compounds the imidazoquinolines also include RSQ, CL075, GDQ and 3M-011 (Fig. 2A). IMQ induced XBP1 splicing in human melanoma cells (Fig. 2B); in contrast, RSQ, CL075 and GDQ were unable to induce ER stress (Figs. 2B and 1C). Treatment with IMQ led to apoptosis of melanoma cells and also potently induced cell-growth arrest (Supplementary Fig. 2A, B). In contrast, RSQ did not induce cell-growth arrest in melanoma cells (Supplementary Fig. 2B). This indicates that the ER-stress inducing and pro-apoptotic activity of IMQ is different from its structurally similar analogs RSQ, GDQ and CL075. Interestingly, the imidazoquinoline 3M-011 could induce XBP1 splicing in melanoma cells suggesting that IMQ and 3M-011 share structural similarities that enable them to induce ER stress in a TLR7-independent manner (Fig. 2C). This structure–activity relationship could be used for developing more potent ER-stress inducing imidazoquinolines with stronger anti-cancer properties.

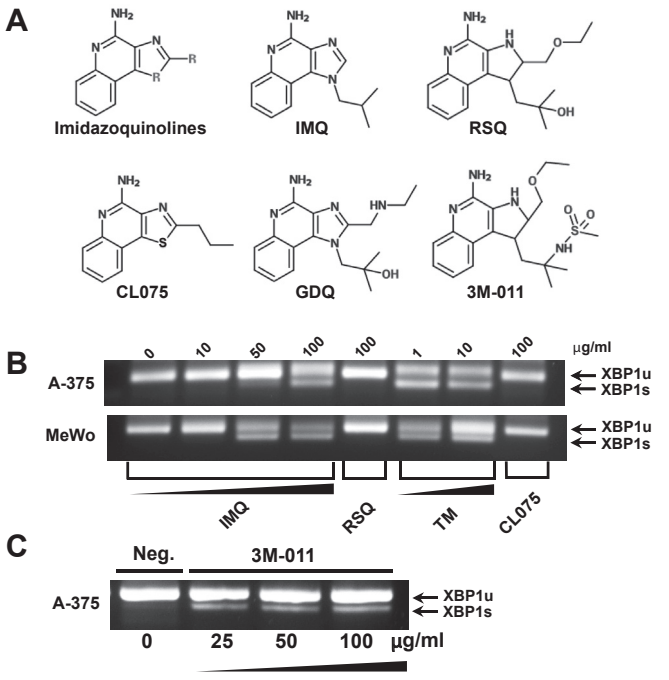

Fig. 2. Structure–activity relationship among imidazoquinolines. IMQ belongs to the imidazoquinolines, a group of small purine analogs that are commonly used as TLR7 or TLR8 agonists (A). After treating A375 melanoma cells with different imidazoquinolines we analyzed their ability to induce XBP1 splicing (B and C). Only IMQ and 3M-011 were able to induce XBP1 splicing indicating that small structural variations among imidazoquinolines affect their ability to induce ER stress.

3.3. IMQ induces the release of Ca^{2+} from intracellular stores independently of TLR7 and IP3R

Since IMQ is a purine analog we hypothesized that it induces influx of extracellular Ca^{2+} in a manner similar to ATP. Indeed, addition of IMQ to melanoma cells induced a fast and transient rise in cytosolic [Ca^{2+}] as measured by Fluo-3-AM (Fig. 3A). Interestingly, neither RSQ nor CL075 induced influx of Ca^{2+}, demonstrating that the Ca^{2+}-inducing property of IMQ is independent of TLR7. To identify Ca^{2+}-associated pathways triggered by IMQ we pre-incubated melanoma cells with a panel of inhibitors followed by addition of IMQ. None of the tested inhibitors could block XBP1 splicing (Supplementary Fig. 3A) or Ca^{2+} influx (data not shown) indicating that IMQ acts independently of extracellular Ca^{2+}, P2X7 channels, phospholipase C (PLC), phosphoinositol 3-kinase (PI3K) and protein kinase C (PKC). At high concentrations the IP3R inhibitor 2-APB (100 μM) inhibited XBP1 splicing and Ca^{2+} influx (data not shown). However, at such high concentrations 2-APB and suffer from severe non-specific effects [22]. Importantly, blocking extracellular Ca^{2+} with 1 mM EGTA did not inhibit XBP1 splicing but revealed that IMQ induced the release of Ca^{2+} from intracellular stores (Fig. 3B). To verify that IMQ induced splicing of XBP1 was independent of store-operated calcium entry we inhibited calcium release-activated channels (CRAC) by pre-treatment of melanoma cells with the CRAC inhibitor BTP-2 (10 μM). Indeed, BPT-2 blocked the influx of extracellular Ca^{2+} without affecting the release of intracellular Ca^{2+} stores (Fig. 3C). Also, blocking CRAC channels with BTP-2 did not block the induction of XBP1 splicing by IMQ (Fig. 3D). This again verified that the ER stress inducing activity of IMQ is independent of extracellular Ca^{2+}. The release of intracellular Ca^{2+} suggests that IMQ leads to opening of IP3R in the ER membrane. Surprisingly, DT40 cells lacking all isoforms of IP3R still responded to IMQ by XBP1 splicing and Ca^{2+} influx (Supplementary Fig. 3B, C) [23]. This indicates that IMQ, in contrast to previous reports, triggers Ca^{2+} influx and ER stress independently of both TLR7 and IP3R [24,25]. Since IMQ is a purine analog and capable of inducing ER stress and influx of Ca^{2+} we hypothesized that it acts via purinergic P2Y or P2X receptors. To test this we treated A375 melanoma cells with different P2X and P2Y agonists (Supplementary Fig. 3D, E). In addition we pre-treated cells with the P2X antagonist suramin to determine if it could inhibit the activity of IMQ (Supplementary Fig. 3E). Natural and synthetic P2X and P2Y agonists were unable to induce XBP1 splicing, and blocking P2X receptors using suramin did not affect the activity of IMQ. This suggests that IMQ might induce XBP1 splicing independently of purinergic receptors. It has been reported that IMQ can signal via adenosine receptors [26].

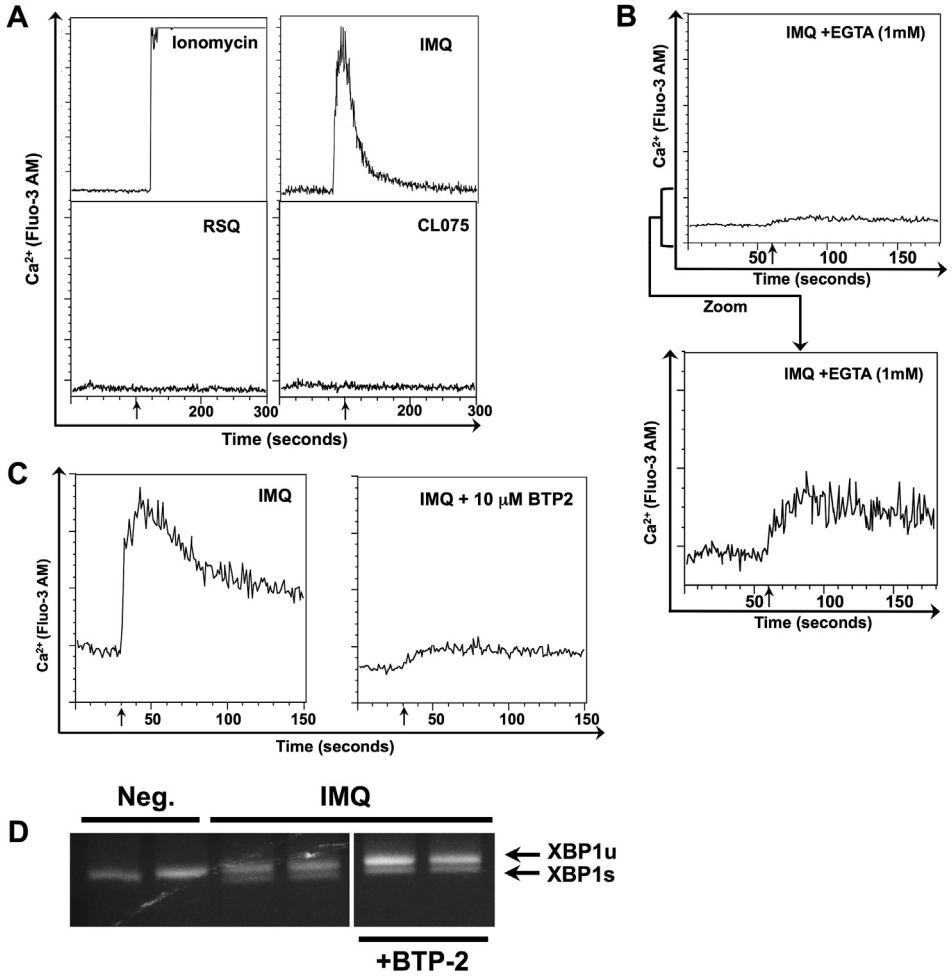

Fig. 3. IMQ induces the release of Ca^{2+} from intracellular stores independently of TLR7 and IP3R. IMQ induced a strong, rapid, and transient increase in cytosolic Ca^{2+} in A375 melanoma cells, while RSQ and CL075 did not affect cytosolic Ca^{2+} levels. Ionomycin was used as a positive control (A). After blocking extracellular Ca^{2+} with 1 mM EGTA we observed a release of Ca^{2+} from intracellular stores after treatment with IMQ (B). To test if IMQ induces XBP1-splicing independently of extracellular Ca^{2+} we blocked CRAC channels with BTP-2 followed by cytosolic Ca^{2+} measurements (C) and XBP1-splicing assays (D). In all, IMQ was still able to induce XBP1 splicing when blocking the entry of extracellular Ca^{2+}.

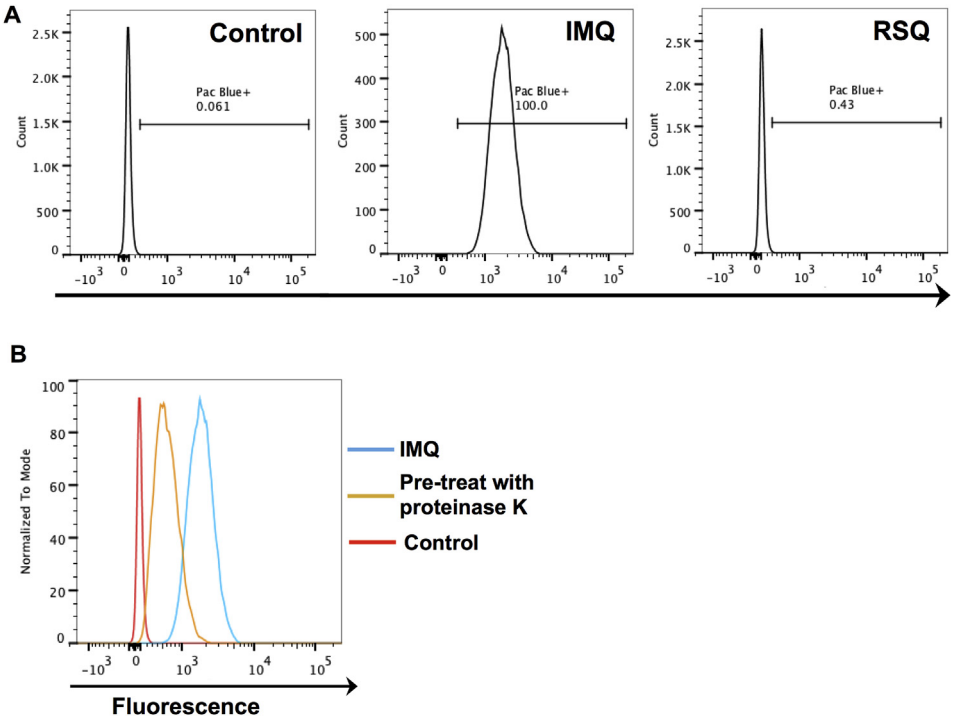

Fig. 4. IMQ binds to proteinase-K sensitive receptors on the surface of melanoma cells. We used the fluorescent properties of IMQ and RSQ to stain A375 melanoma cells followed by flow cytometry using excitation with an ultraviolet laser (355 nm) and detection using the 440/40 filter set. Binding of IMQ to A375 melanoma cells was readily detected after flow cytometry (A). In contrast, RSQ was unable to stain the cells. Pre-treatment of melanoma cells with proteinase K reduced the binding of IMQ indicating that IMQ induces ER-stress by binding to at least one protein-based cell-surface receptor (B).

However, adenosine could not induce ER stress in human melanoma cells (Supplementary Fig. 3F, G). In all, IMQ induces the influx of Ca^{2+} via an unknown mechanism and we suggest that IMQ induces ER stress via depletion of Ca^{2+} from the ER.

3.4. IMQ binds to a proteinase-K sensitive receptor on the surface of melanoma cells

Since the fluorescent properties of IMQ and RSQ previously have been used to measure release of IMQ from topical formulations and the encapsulation efficiency of RSQ we asked if these properties could be exploited for staining A375 melanoma cells with IMQ and RSQ. For IMQ it has experimentally been determined that the $excitation_{max}$ = 235 nm and $emission_{max}$ = 341 nm [27]. Excitation = 260 nm and emission = 360 nm has successfully been used for fluorescent detection of RSQ [28]. Addition of IMQ to A375 melanoma cells stained the cells as detected by flow cytometry using an ultraviolet laser (355 nm) and the Pacific Blue (440/40) filter setting (Fig. 4A). In contrast, RSQ were unable to stain A375 melanoma cells using the same approach. This strongly suggests that a non-TLR7 receptor for IMQ is expressed on A375 melanoma cells and that this receptor is not bound by RSQ. To verify that the effect of IMQ is mediated via a cell surface receptor, we pre-treated the melanoma cells with proteinase K followed by washing and addition of IMQ. Indeed, proteinase-K treatment clearly inhibited the binding of IMQ (Fig. 4B). In all, our data suggests that IMQ binds a non-purinergic receptor that triggers the influx of Ca^{2+} and ER stress.

4. Discussion

IMQ has been successfully used as an immunostimulatory molecule for almost 30 years. Even though the identification of TLR7 as the receptor for IMQ clarified many of its immunostimulatory properties, it is evident that imiquimod also have TLR7- and MyD88-independent effects. Many cell types lacking TLR7 and TLR8 still respond to IMQ, and topical treatment of *Tlr7*$^{-/-}$ and *Myd88*$^{-/-}$ mice with IMQ nevertheless induces strong reactions in skin [17,18]. Thus the role of IMQ as a potent inducer of ER stress and apoptosis might explain some of the TLR7-independent effects of IMQ. In stark contrast to its close analogs RSQ, CL075 and GDQ, treatment with IMQ leads to XBP1 splicing and the induction of ER-stress target genes. This is a surprising finding given the close structural similarities between IMQ, RSQ, CL075 and GDQ, and could potentially be used for structure–activity relationship studies to obtain new more potent ER-stress inducers. In addition to IMQ, we also identified the imidazoquinoline 3M-011 as an inducer of XBP1 splicing suggesting that new imidazoquinoline variants with greater ER-stress inducing activity could be generated. To our knowledge IMQ is the third FDA approved drug that induces ER stress, the others being the anti-cancer drug bortezomib and the anti-HIV drug nelfinavir. Since nelfinavir, bortezomib and IMQ are effective against certain forms of cancer, it appears that ER-stress inducers in general could be effective anti-cancer drugs. This motivates a search for additional ER-stress inducers. While the proteasome inhibitor bortezomib triggers ER-stress via the induction of protein misfolding in the ER, we demonstrate that IMQ appears to induce ER stress via depletion of Ca^{2+} from the ER. IMQ induces a rapid depletion of Ca^{2+} from the ER and influx of extracellular Ca^{2+}. Identifying the unknown IMQ receptor could potentially lead to the discovery of new physiological pathways for induction of ER stress, and to development of new more potent pharmacological inducers of ER stress.

Acknowledgments

This work was funded by the Swedish Research Council (VR) (K2013-99X-22235-01-5); the Center of excellence for research on

inflammation and cardiovascular disease (CERIC); and Karolinska Institutet, Sweden. The authors thank Professor Laurie Glimcher for valuable scientific input. The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2016.03.080.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.03.080.

References

[1] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[2] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell 107 (2001) 881–891.
[3] A.H. Lee, N.N. Iwakoshi, L.H. Glimcher, XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response, Mol. Cell. Biol. 23 (2003) 7448–7459.
[4] M. Maurel, E.P. McGrath, K. Mnich, S. Healy, E. Chevet, A. Samali, Controlling the unfolded protein response-mediated life and death decisions in cancer, Semin. Cancer Biol. 33 (2015) 57–66.
[5] A. Fribley, Q. Zeng, C.Y. Wang, Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells, Mol. Cell. Biol. 24 (2004) 9695–9704.
[6] E.A. Obeng, L.M. Carlson, D.M. Gutman, W.J. Harrington Jr., K.P. Lee, L.H. Boise, Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells, Blood 107 (2006) 4907–4916.
[7] J.J. Gills, J. Lopiccolo, J. Tsurutani, R.H. Shoemaker, C.J. Best, M.S. Abu-Asab, J. Borojerdi, N.A. Warfel, E.R. Gardner, M. Danish, M.C. Hollander, S. Kawabata, M. Tsokos, W.D. Figg, P.S. Steeg, P.A. Dennis, Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis in vitro and in vivo, Clin. Cancer Res. 13 (2007) 5183–5194.
[8] P. Pyrko, A. Kardosh, W. Wang, W. Xiong, A.H. Schonthal, T.C. Chen, HIV-1 protease inhibitors nelfinavir and atazanavir induce malignant glioma death by triggering endoplasmic reticulum stress, Cancer Res. 67 (2007) 10920–10928.
[9] F. Martinon, X. Chen, A.H. Lee, L.H. Glimcher, TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages, Nat. Immunol. 11 (2010) 411–418.
[10] S. Savic, L. Ouboussad, L.J. Dickie, J. Geiler, C. Wong, G.M. Doody, S.M. Churchman, F. Ponchel, P. Emery, G.P. Cook, M.H. Buch, R.M. Tooze, M.F. McDermott, TLR dependent XBP-1 activation induces an autocrine loop in rheumatoid arthritis synoviocytes, J. Autoimmun. 50 (2014) 59–66.
[11] A. Afrazi, M.F. Branca, C.P. Sodhi, M. Good, Y. Yamaguchi, C.E. Egan, P. Lu, H. Jia, S. Shaffiey, J. Lin, C. Ma, G. Vincent, T. Prindle Jr., S. Weyandt, M.D. Neal, J.A. Ozolek, J. Wiersch, M. Tschurtschenthaler, C. Shiota, G.K. Gittes, T.R. Billiar, K. Mollen, A. Kaser, R. Blumberg, D.J. Hackam, Toll-like receptor 4-mediated endoplasmic reticulum stress in intestinal crypts induces necrotizing enterocolitis, J. Biol. Chem. 289 (2014) 9584–9599.
[12] S. Toosi, S.J. Orlow, P. Manga, Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL6 and IL8, J. Investig. Dermatol. 132 (2012) 2601–2609.
[13] M.R. Farrukh, U.A. Nissar, Q. Afnan, R.A. Rafiq, L. Sharma, S. Amin, P. Kaiser, P.R. Sharma, S.A. Tasduq, Oxidative stress mediated Ca(2+) release manifests endoplasmic reticulum stress leading to unfolded protein response in UV-B irradiated human skin cells, J. Dermatol. Sci. 75 (2014) 24–35.
[14] K. Sugiura, Unfolded protein response in keratinocytes: impact on normal and abnormal keratinization, J. Dermatol. Sci. 69 (2013) 181–186.
[15] J.F. Gerster, K.J. Lindstrom, R.L. Miller, M.A. Tomai, W. Birmachu, S.N. Bomersine, S.J. Gibson, L.M. Imbertson, J.R. Jacobson, R.T. Knafla, P.V. Maye, N. Nikolaides, F.Y. Oneyemi, G.J. Parkhurst, S.E. Pecore, M.J. Reiter, L.S. Scribner, T.L. Testerman, N.J. Thompson, T.L. Wagner, C.E. Weeks, J.D. Andre, D. Lagain, Y. Bastard, M. Lupu, Synthesis and structure-activity-relationships of 1H-imidazo[4,5-c]quinolines that induce interferon production, J. Med. Chem. 48 (2005) 3481–3491.
[16] H. Hemmi, T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda, S. Akira, Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway, Nat. Immunol. 3 (2002) 196–200.
[17] A. Walter, M. Schafer, V. Cecconi, C. Matter, M. Urosevic-Maiwald, B. Belloni, N. Schonewolf, R. Dummer, W. Bloch, S. Werner, H.D. Beer, A. Knuth, M. van den Broek, Aldara activates TLR7-independent immune defence, Nat. Commun. 4 (2013) 1560.
[18] S.J. Kim, G.H. Park, D. Kim, J. Lee, H. Min, E. Wall, C.J. Lee, M.I. Simon, S.J. Lee, S.K. Han, Analysis of cellular and behavioral responses to imiquimod reveals a unique itch pathway in transient receptor potential vanilloid 1 (TRPV1)-expressing neurons, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 3371–3376.
[19] A. Celli, D.S. Mackenzie, D.S. Crumrine, C.L. Tu, M. Hupe, D.D. Bikle, P.M. Elias, T.M. Mauro, Endoplasmic reticulum Ca2+ depletion activates XBP1 and controls terminal differentiation in keratinocytes and epidermis, Br. J. Dermatol. 164 (2011) 16–25.
[20] M.C. Lebre, A.M. van der Aar, L. van Baarsen, T.M. van Capel, J.H. Schuitemaker, M.L. Kapsenberg, E.C. de Jong, Human keratinocytes express functional toll-like receptor 3, 4, 5, and 9, J. Investig. Dermatol. 127 (2007) 331–341.
[21] G. Kollisch, B.N. Kalali, V. Voelcker, R. Wallich, H. Behrendt, J. Ring, S. Bauer, T. Jakob, M. Mempel, M. Ollert, Various members of the Toll-like receptor family contribute to the innate immune response of human epidermal keratinocytes, Immunology 114 (2005) 531–541.
[22] M.D. Bootman, T.J. Collins, L. Mackenzie, H.L. Roderick, M.J. Berridge, C.M. Peppiatt, 2-aminoethoxydiphenyl borate (2-APB) is a reliable blocker of store-operated Ca2+ entry but an inconsistent inhibitor of InsP3-induced Ca2+ release, FASEB J. 16 (2002) 1145–1150.
[23] H. Sugawara, M. Kurosaki, M. Takata, T. Kurosaki, Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor, EMBO J. 16 (1997) 3078–3088.
[24] H. Hwang, H. Min, D. Kim, S.W. Yu, S.J. Jung, S.Y. Choi, S.J. Lee, Imiquimod induces a Toll-like receptor 7-independent increase in intracellular calcium via IP(3) receptor activation, Biochem. Biophys. Res. Commun. 450 (2014) 875–879.
[25] A. El-Khattouti, D. Selimovic, M. Hannig, E.B. Taylor, Z.Y. Abd Elmageed, S.Y. Hassan, Y. Haikel, E. Kandil, M. Leverkus, R.T. Brodell, M. Megahed, M. Hassan, Imiquimod-induced apoptosis of melanoma cells is mediated by ER stress-dependent Noxa induction and enhanced by NF-kappaB inhibition, J. Cell. Mol. Med. 20 (2016) 266–286.
[26] M.P. Schon, M. Schon, Imiquimod: mode of action, Br. J. Dermatol. 157 (Suppl. 2) (2007) 8–13.
[27] B. Zhao, Y.Z. Rong, X.H. Huang, J.S. Shen, Experimental and theoretical study on the structure and electronic spectra of imiquimod and its synthetic intermediates, Bioorg. Med. Chem. Lett. 17 (2007) 4942–4946.
[28] A.D. Duong, S. Sharma, K.J. Peine, G. Gupta, A.R. Satoskar, E.M. Bachelder, B.E. Wyslouzil, K.M. Ainslie, Electrospray encapsulation of toll-like receptor agonist resiquimod in polymer microparticles for the treatment of visceral leishmaniasis, Mol. Pharm. 10 (2013) 1045–1055.