Cyclin-dependent kinase 2 inhibitor SU9516 increases sensitivity of colorectal carcinoma cells Caco-2 but not HT29 to BH3 mimetic ABT-737

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Abstract. Colorectal carcinoma (CRC) that represents one of the major causes for cancer related death in humans is often associated with over-expression of anti-apoptotic proteins of Bcl-2 family. The aim of presented study was to determine the effect of ABT-737 inhibitor of anti-apoptotic proteins Bcl-2, Bcl-X_L and Bcl-w as well as cyclin-dependent kinase 2 (CDK2) inhibitor SU9516 alone and in combination with ABT-737 on survival of colorectal cell lines HT29 and Caco-2. We have shown that both Caco-2 and HT29 cells that are relatively resistant to ABT-737 are also partially sensitive to SU9516, which increased sensitivity of Caco-2 but not HT29 cells to ABT-737. Increased sensitivity of Caco-2 cells to ABT-737 after addition of SU9516 correlated well with SU9516-induced decrease of Mcl-1 expression while we have not observed downregulation of Mcl-1 after the treatment of HT29 cells with SU9516. Instead of this, we have shown that treatment of HT29 cells with SU9516 is associated with decreased expression of tumour suppressor protein p53. Our findings provide a rationale for clinical use of Bcl-2 family inhibitors in combination with CDK2 inhibitors for treatment of Mcl-1-dependent colorectal tumours associated with expression of Bcl-2, Bcl-X_L and Bcl-w proteins. In addition, we have shown potential of CDK2 inhibitors for treatment of tumours expressing R273H mutant p53.

Key words: Cell death — Apoptosis — Bcl-2 family proteins — Cyclin-dependent kinase — Colorectal carcinoma

Introduction

Colorectal carcinoma (CRC) represents one of the major causes for cancer-related death in humans (Ferlay et al. 2010). Even efficiency of novel and targeted therapeutic approaches that are rapidly emerging is not high enough and the prognosis in the metastatic stage (UICC IV) is poor (Lansdorp-Vogelaar et al. 2009).

In addition to different molecular hallmarks of CRC (Dienstmann et al. 2017), it has been documented that anti-apoptotic proteins of Bcl-2 family Bcl-w (Wilson et al. 2000) and Bcl-X_L (Krajewska et al. 1996; Zhang et al.

2008a; Scherr et al. 2016) are frequently over-expressed in colorectal carcinomas and patients with high Bcl-X_L expression showed significantly poorer overall survival than those with low Bcl-X_L expression (Jin-Song et al. 2011). Proteins of Bcl-2 family essential regulators of mitochondrial apoptosis (Czabotar et al. 2014; Hatok and Racay 2016) are emerging interests in development of new anticancer agents and several small molecules with a potential of binding to anti-apoptotic proteins of Bcl-2 family were developed during past 10 years (Delbridge et al. 2016; Ashkenazi et al. 2017; Schenk et al. 2017). ABT-737 is a small BH3-only mimetic molecule that binds to the anti-apoptotic Bcl-2 family proteins Bcl-2, Bcl-X_L, and Bcl-w (Oltersdorf et al. 2005). Binding of ABT-737 to Bcl-2, Bcl-X_L, and Bcl-w disrupts their anti-apoptotic functions and is associated with death of various tumour cells (Chauhan et al. 2007; Placzek et al. 2010). ABT-737-induced cell death exhibited all the

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characteristic ultrastructural changes of apoptosis induced by activation of caspase-9 and was completely inhibited in cells deficient for Bax/Bak or caspase-9 (Vogler et al. 2009).

Since ABT-737 exhibits minimal affinity to another important anti-apoptotic member of Bcl-2 family Mcl-1 (Oltersdorf et al. 2005), sensitivity of tumour cells to ABT-737 depends significantly on the relative expression levels of Bcl-2/ Bcl-X$_L$ *versus* Mcl-1 (Konopleva et al. 2006; van Delft et al. 2006) or Mcl-1 *versus* BIM and PUMA (Pétigny-Lechartier et al. 2017). In agreement with this, down-regulation of Mcl-1 expression significantly enhances ABT-737 cytotoxicity (Chen et al. 2007; Lin et al. 2007; Lestini et al. 2009). On the other hand, ABT-737 displaces BIM from the BH3-binding pocket of Bcl-2 that is associated with BIM-mediated activation of Bax and consequent initiation of mitochondrial apoptosis (Del Gaizo Moore et al. 2007). It seems that the extent of Bcl-2 bound to BIM, rather than total Bcl-2 expression levels, may determine cellular sensitivity to ABT-737 (Deng et al. 2007). In hand with this, ABT-737 has been shown to interact with certain anticancer agents capable of up-regulating BIM (Kuroda et al. 2006; Zhang et al. 2008b; Stefaniková et al. 2013).

Inhibitors of cyclin-dependent kinase 2 (CDK2) including SU9516 exhibit also significant potential with respect to treatment of different types of malignant diseases (Asghar et al. 2015; Chohan et al. 2015) including CRC (Yamamoto et al. 1998). Down-regulation of Mcl-1 expression (Gao et al. 2006), down-regulation and phosphorylation of Mcl-1 associated with consequent release of BIM bound to Mcl-1 (Choudhary et al. 2015) as well as caspase-3 activation (Lane et al. 2001; Gao et al. 2006) were shown to be among the mechanisms involved in the cell death induction by CDK2 inhibitors.

The aim of presented study was to determine effect of ABT-737 as well as SU9516 alone and in combination with ABT-737 on survival of colorectal cell lines HT29 and Caco-2. In addition, we have also focused our interest on the RT-PCR and Western blot analysis of the levels of selected proteins of Bcl-2 family in untreated HT29 and Caco-2 cells as well as on the analysis of the impact of SU9516 on the levels of proteins of Bcl-2 family in the cells treated with SU9516 alone and in combination with ABT-737.

Material and Methods

SU9516 (Calbiochem), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium dodecyl-sulphate (SDS) were from Sigma Aldrich, ABT-737 was from Abbott Laboratories. In this study were used rabbit polyclonal antibodies against Mcl-1 (SAB4501843) from Sigma-Aldrich, Bcl-X$_L$ (sc-7195), BIM (sc-11425) and BAX (sc-439). Mouse monoclonal antibodies against p53

(SC-5576) and β-actin (SC-47778); mouse anti-rabbit (SC-2357) and goat anti-mouse (SC-2005) were from Santa Cruz Biotechnology, secondary antibodies conjugated with horseradish peroxidase.

Cell culture

The HT29 and Caco-2 cell lines were purchased from ATCC. HT29 cells were grown at 37°C/5% CO$_2$ in McCoy´s 5a medium (Sigma-Aldrich) supplemented with 10% foetal calf serum and 1% penicillin-streptomycin (all PAA). Caco-2 cells were grown at 37°C/5% CO$_2$ in MEM–Earle (Sigma-Aldrich) containing nonessential amino acids and 2 mM L-glutamine supplemented with 20% foetal calf serum and 1% penicillin-streptomycin (all PAA).

Cell viability assay

Both HT29 and Caco-2 cells were seeded in 96-well plates at concentrations of 4×10^5 *per* ml in 2×10^6 Caco-2 cells *per* ml in triplicate wells and were incubated 24–72 h with or without various concentrations of ABT-737 and/or SU9516. At the end of incubation the absorbance of formazan that resulted from oxidation of added MTT by vital cells was determined spectrophotometrically using microplate reader BioRad 2010. The relative viability of the cells was determined as ratio of optical density of formazan produced by treated cells to optical density of formazan produced by non-treated control cells and expressed as per cent of control. The cell growth was determined as time course of optical density of formazan produced by particular cell population.

Isolation of total RNA and quantitative real time reverse-transcription polymerase chain reaction (qRT-PCR) based on TaqMan® array

Total RNA was isolated from harvested cells using Tri reagent (Sigma-Aldrich) following the manufacturer's protocol. Total RNA (5 µg) was reversely transcribed to cDNA using Maxima First Strand cDNA synthesis kit for RT-qPCR (Thermo Scientific) kit according to protocol supplied by manufacturer.

In our experiments, the predesigned 384-well microfluidic cards (TaqMan® array) with eight sample loading ports were used. The cards were configured into four identical 96-gene sets containing assays for 93 human apoptosis-related genes in addition to three endogenous controls (*18S RNA, actin* and *GAPDH*). The 93 genes were categorized into multiple target classes including genes coding for proteins involved in intrinsic, extrinsic, regulatory, and execution apoptosis traits. A reaction mixture prepared according to protocol supplied by manufacturer containing cDNA

template (100 ng) and a particular volume of TaqMan_ universal master mix (Applied Biosystems) was immediately loaded into each line of the TLDA microfluidic card. Each card was centrifuged twice at 1,200 rpm for 1 min to distribute the PCR mix into the wells of the card, before it was sealed and loaded into the ViiA™ 7 Real-Time PCR System (Life Technologies). The thermal cycle conditions and the threshold cycle, C_T, were automatically assigned by the SDS2.2 software package (Life Technologies).

Array normalization and selection of differentially expressed genes

The arithmetic standard normalization procedures recommended by the Data Assist software for microarray data were followed. Data transformation was corrected for the signal from the endogenous control (*ACTB*). The relative changes in gene expression between cells were analysed using the $2^{-\Delta\Delta C_T}$ method (Schmittgen and Livak 2008) where $\Delta\Delta CT = (C_{Ttarget} - C_T)_{HT29\ cells} - (C_{Ttarget} - C_T)_{Caco-2\ cells}$. Relative quantities (RQ) were determined using the equation $RQ = 2^{-\Delta\Delta C_T}$. All data were generated in triplicate (different TLDA plates) and expressed as the mean fold change in expression of the particular gene in HT29 cells relative to the mean of the Caco-2 cells. Differentially expressed genes were selected from the normalized data. Transcription of particular gene was considered to be significant when the

average fold change was less than 0.5 for downregulated genes or more than 1.5 for upregulated genes, and statistically significant when the corresponding *p* value was less than 0.05.

Western blotting

Isolation of proteins was performed by extraction with TriReagent (Invitrogen) according to manufacturer's instructions. Extracted proteins were separated on 12% SDS-polyacrylamide gels under reducing conditions with 30 μg of protein loaded *per* lane, transferred to nitrocellulose membrane *via* semidry transfer and probed with antibodies specific to p53, Mcl-1, Bcl-X_L, BIM, BAX and β-actin. After incubation of membranes with particular secondary antibodies, immunopositive bands were visualized using the chemiluminiscent substrate SuperSignal West Pico (Thermo Scientific) and Chemidoc XRS system (BioRad). Specific bands were documented by Quantity One software (BioRad).

Statistical analysis

All statistical analyses were done using GrafPhad InStat V2.04a (GrafPhad Software). The unpaired Tukey's test was used to determine differences between viability of control and treated cells. Significance level was set at *p* < 0.05.
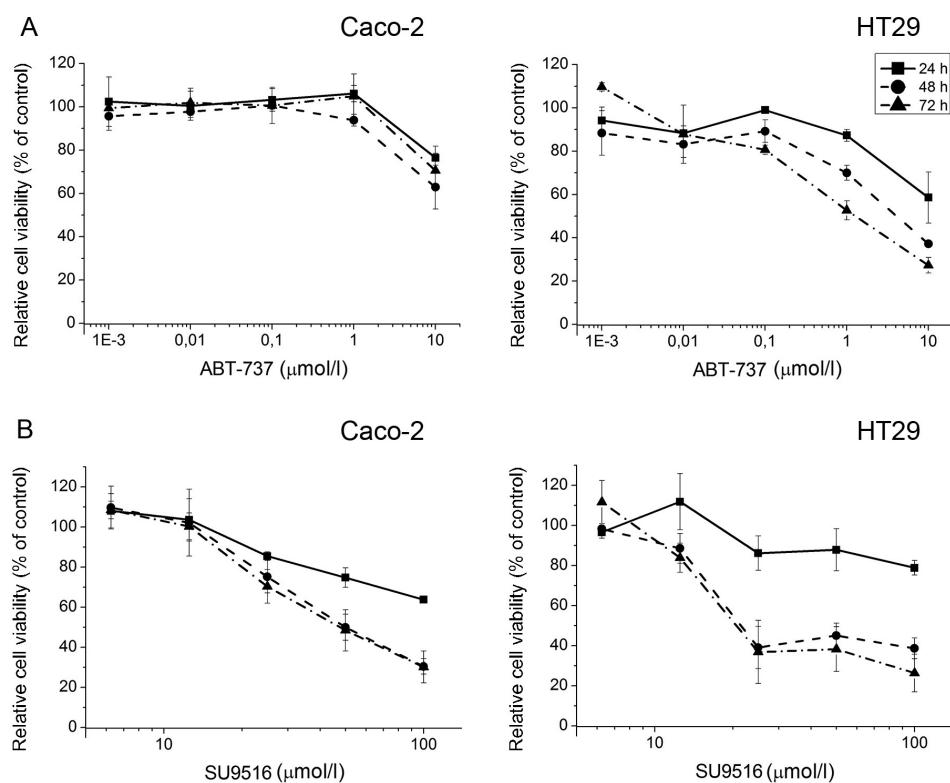

Figure 1. The effect of ABT-737 (**A**) and SU9516 (**B**) on relative viability of Caco-2 and HT29 cells. HT29 and Caco-2 cells were treated with indicated concentrations of ABT-737 and SU9516 for 24, 48, and 72 h and then the relative cell viability was determined by MTT test as described in Material and Methods. Data are presented as means ± SEM (three independent experiments performed in triplicate *per* each cell line).

Results

Treatment of the cells with ABT-737 for 24, 48 and 72 h has revealed time- and concentration-dependent impact of ABT-737 on relative viability of both Caco-2 and HT29 cells (Fig. 1A). The impact of ABT-737 on relative viability of Caco-2 cells was faster and decrease of relative cell viability was already observed after 24 h of incubation. However, Caco-2 cells were relative resistant and the relative viability of Caco-2 cells at concentration of ABT-737 10 µmol/l was 76.5 ± 5.3 % ($p > 0.05$), 62.9 ± 10.1 % ($p > 0.05$) and 70.6 ± 7.9% ($p < 0.05$) after 24, 48 and 72 h, respectively. In contrary, significant decrease of relative viability of HT29 cells treated with ABT-737 was observed after 48 h but HT29 cells were significantly more sensitive to ABT-737. The LC_{50} values for ABT-737 were estimated to be 6.41 ± 0.6 and 2.5 ± 1.6 µmol/l after 48 and 72 h, respectively.

The impact of SU9516 on relative viability of HT29 and Caco-2 cells was also time- and concentration-dependent (Fig. 1B). Caco-2 cells were also less sensitive to SU9516 and the LC_{50} values for SU9516 were estimated to be 49.9 ± 6.4, and 49.9 ± 5.1 µmol/l after 48 and 72 h, respectively. SU9516 in concentrations 25, 50 and 100 µmol/l led to the significant decrease of relative viability of HT29 cells which was dominant after 48 h of incubation. The LC_{50} values for SU9516 were estimated to be 22.2 ± 3.6 and 21.5 ± 4.1 µmol/l after 48 and 72 h, respectively.

In order to test effect of SU9516 in combination with ABT-737 fixed concentrations of SU9516 (12.5 µmol/l) were used whereas concentrations of ABT-737 were varied in the complete investigated range. SU9516 in concentration 12.5 µmol/l did not exhibit significant impact on relative viability of Caco-2 cells, while the same concentration decreased relative viability of HT29 cells to 88.6 ± 7.4% ($p >$
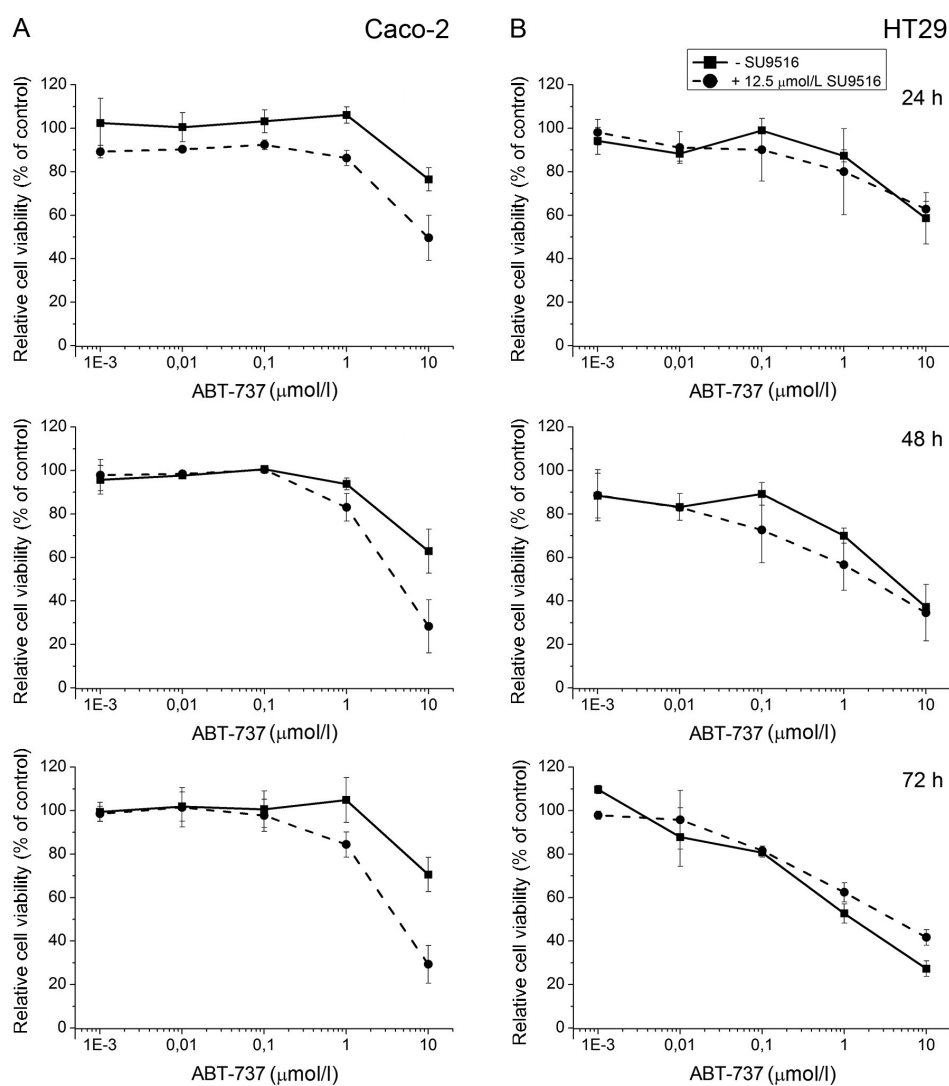

Figure 2. The effect of ABT-737 in combination with 12.5 µmol/l SU9516 on relative viability of Caco-2 (**A**) and HT29 (**B**) cells. Caco-2 and HT29 cells were treated with indicated concentrations of ABT-737 in combination with 12.5 µmol/l SU9516 for 24, 48, and 72 h and then the relative cell viability was determined by MTT test as described in Material and Methods. Data are presented as means ± SEM (three independent experiments performed in triplicate *per* each cell line).
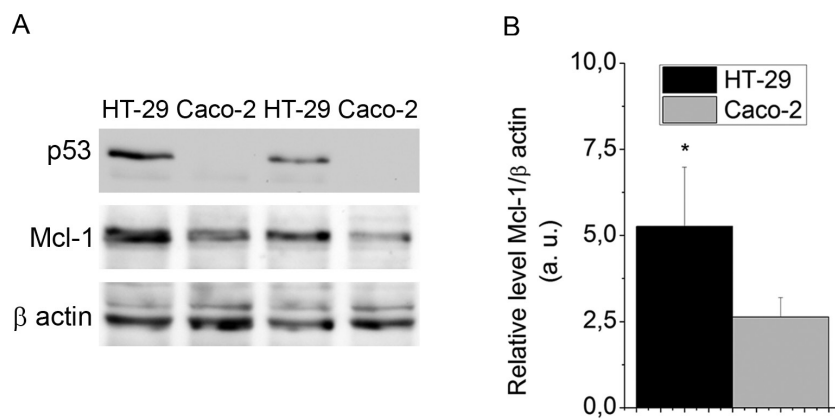
A

B

Figure 3. Levels of p53 and Mcl-1 in untreated HT29 and Caco-2 cells. Total cell extracts were prepared from both untreated HT29 and Caco-2 cells. The levels of p53 and Mcl-1 were evaluated by Western blot analysis of total cell extracts as described in Materials and Methods. Data are presented as means ± SD (4 independent experiments per each cell line). * $p < 0.05$, (Tukey's test to determine differences between levels of particular proteins in both types of cells).

0.05) and 83.9 ±7.2% ($p < 0.05$) after 48 and 72 h, respectively. We have observed significant difference in the effect of ABT-737 on relative viability of Caco-2 cells incubated in the absence or presence of 12.5 μmol/l SU9516 (Fig. 2A) for 24, 48 and 72 h. The relative viability of Caco-2 cells incubated in the presence of 12.5 μmol/l of SU9516 was significantly decreased at concentrations of ABT-737 10 μmol/l as compared to the relative viability of Caco-2 cells incubated with ABT-737 only (28.3 ± 12.2% *versus* 62.9 ± 10.1% after 48 h of incubation and 29.3 ± 8.7% *versus* 70.6 ± 7.9% after 72 h of incubation). In HT29 cells, addition of SU9516 did not affect the cytotoxic effect of ABT-737 (Fig. 2B).

In order to compare gene expression profile of genes coding for Bcl-2 family proteins, total RNA isolated from non-treated HT29 and Caco-2 cells was analysed by qRT-PCR based on TaqMan® array human apoptosis panel. The results of qRT-PCR analysis showing only significantly different expressed genes are presented in Table 1. In HT29 cells, significantly higher gene expression of pro-apoptotic/anti-survival (BAX, BOK, BID, BIM, PUMA, Noxa) proteins of Bcl-2 family was observed. In the same cells, we have also documented significantly higher expression of gene coding for anti-survival protein Bcl-Rambo involved in induction of mitophagy (Murakawa et al. 2015). In Caco-2 cells,

significantly higher gene expression of one pro-apoptotic/anti-survival Bcl-2L14 (Bcl-g) protein of Bcl-2 family was observed.

In addition to the mRNA analysis, we have focused our interests on Western blot detection of selected proteins of Bcl-2 family, playing a role in sensitivity and resistance of malignant cells to ABT-737, in non-treated HT29 and Caco-2 cells. Despite no changes observed by mRNA analysis, significantly higher level of Mcl-1 protein (196.9% of Mcl-1 in Caco-2 cells, $p < 0.05$) was detected in HT29 cells (Fig. 3). The levels of other investigated Bcl-2 family proteins (Bcl-X_L, BIM and BAX) were not significantly different (results not shown). In addition to proteins of Bcl-2 family we have analysed expression of tumour suppressor protein p53 that was detected in HT29 cells but not in Caco-2 cells.

We have also investigated the effect of SU9516 alone and in combination with ABT-737 on expression of Mcl-1 and p53. As shown on Fig. 4, incubation of Caco-2 cells with either SU9516 or SU9516 in combination with ABT-737 was associated with decrease in Mcl-1 protein level observed after 48 and 72 h incubation. The level of Mcl-1 protein was unaltered in HT29 cells treated with SU9516 alone or in combination with ABT-737. However, we have observed

Table 1. Gene expression profiling of anti-survival proteins of Bcl-2 family in HT29 and Caco-2 cells

| Gene name	ENSEMBL gene number	Fold change	*p*	Gene description
BAX	ENSG00000087088	3.4	0.0007	*BCL2*-associated X protein, *BAX*
BOK	ENSG00000176720	14.4	0.0018	*BCL2*-related ovarian killer
BID	ENSG00000015475	3.5	0.0068	*BH3* interacting domain death agonist
BBC3	ENSG00000105327	33.8	0.0002	*BCL2* binding component 3, PUMA
PMAIP1	ENSG00000141682	21.1	0.0006	phorbol-12-myristate-13-acetate-induced protein 1, Noxa
BCL2L11	ENSG00000153094	2	0.0098	*BCL2-like 11*, *BIM*
BCL2L13	ENSG00000099968	3.3	0.0022	*BCL2-like 13*, *Bcl-Rambo*
BCL2L14	ENSG00000121380	0.53	0.0002	*BCL2-like 14*, *Bcl-g*
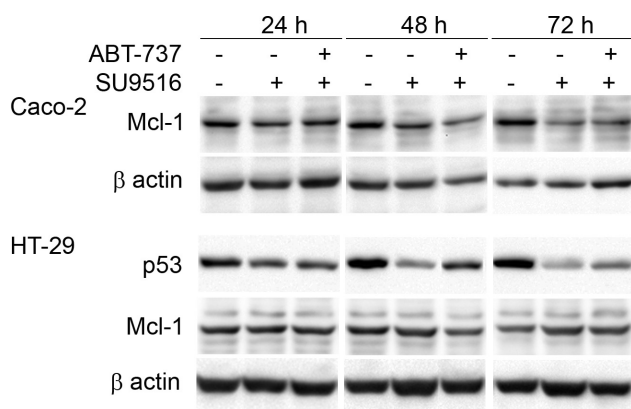
Figure 4. The effect of SU9516 alone and in combination with ABT-737 on the level of Mcl-1. Total cell extracts were prepared from both HT29 and Caco-2 cells after the treatment with either 12.5 µmol/l or 12.5 µmol/l of SU9516 in combination with 10 µmol/l ABT-737 for 24, 48 and 72 h. The effect of treatments on the levels of p53 and Mcl-1 was evaluated by Western blot analysis of total cell extracts as described in Material and Methods.

decreased expression of p53 protein in HT29 cells treated with SU9516 alone or in combination with ABT-737 after 48 and 72 h (Fig. 4).

Discussion

In this study, we have shown that both Caco-2 and HT29 cells are relatively resistant to ABT-737 as well as that CDK2 inhibitor SU9516 despite inducing cell death of both CRC cell lines used in the study increases sensitivity of Caco-2 but not HT29 cells to ABT-737.

The relative resistance of both Caco-2 and HT29 cells to ABT-737 correlated well with the fact that both HT29 and Caco-2 cells are negative for expression of Bcl-2 (on both mRNA and protein level, data not shown) since Bcl-2 was shown to be a better ABT-737 target than Bcl-X_L or Bcl-w (Rooswinkel et al. 2012). In agreement, the CRC cells HCT116, expressing significant amounts of Bcl-2 protein and the same level of Bcl-X_L protein as HT29 cells, were much more sensitive to ABT-737 than Bcl-2 negative HT29 cells (Okumura et al. 2008). We have also shown the higher sensitivity of HT29 cells than Caco-2 cells to ABT-737 that was in agreement with previously published data (Koehler et al. 2014).

In addition to ABT-737, we have also documented different sensitivity of Caco-2 and HT29 cells to CDK2 inhibitor SU9516. Caspase-3-dependent mitochondrial apoptosis has been documented as the major mechanism of SU9516-induced death of tumour cells (Gao et al. 2006) including HT29 cells (Lane et al. 2001). In agreement, we have

documented down-regulation of Mcl-1 protein in Caco-2 since down-regulation of Mcl-1 expression or inhibition of Mcl-1 function have been shown to be associated with caspase-3-dependent apoptosis initiation and consequent death of different types of cancer cells (for review see Belmar and Fesik 2015). In HT29 cells, we have not observed down-regulation of Mcl-1 after the treatment with SU9516. Instead of this, we have shown that treatment of HT29 cells with SU9516 was associated with decreased expression of tumour suppressor protein p53. In normal cells, wild type tumour suppressor protein p53 is quickly eliminated and its expression is stabilised after damage to DNA or other intracellular stress conditions (Bullock and Fersht 2001) while in tumour cells p53 is frequently mutated and the mutation have different impact on p53 function and stability (Muller and Vousden 2013). Tumour cell bearing some nonsense mutations of p53 including Caco-2 cells (Liu and Bodmer 2006) do not express p53 protein while the cells expressing some missense mutations of p53 that are highly frequent in CRC (Soussi and Wiman 2015) are characterised by over-expression of mutated p53 (for review see Brosh and Rotter 2009). We have shown stable expression of p53 protein in HT29 cells that are expressing p53 bearing R273H missense mutation (Leroy et al. 2014). Such mutation is associated with gain of function of p53 protein leading to increased resistance of the tumour cells to chemotherapy (Wong et al. 2007), increased invasion and metastatic progression of the tumour cells (Oren and Rotter 2010) including HT29 (Muller et al. 2013). In addition, it has been shown that knockdown of R273H mutant p53 is associated with increased sensitivity of tumour cells to chemotherapy *via* increase of procaspase-3 expression and activation (Wong et al. 2007).

As mentioned above, sensitivity of the tumour cells to ABT-737 can be increased by down-regulation of Mcl-1 (Chen et al. 2007; Lin et al. 2007) and consequent release of Mcl-1 from the binding to BIM protein (Mazumder et al. 2012; Choudhary et al. 2015) that can be achieved by the treatment of some cells with inhibitors of CDK2 including SU9516 (Gao et al. 2006). Despite low sensitivity of Caco-2 cells to SU9516, combination of SU9516 with ABT-737 was associated with increased sensitivity of Caco-2 cells to ABT-737 while sensitivity of HT29 cells to ABT-737 was unaltered after the treatment of HT29 cells with ABT-737 in combination with SU9516. Our results are consistent with a view that increased sensitivity of Caco-2 cells to ABT-737 after addition of SU9516 is related to SU9516-induced decrease of Mcl-1 expression since our Western blot experiments revealed decrease of Mcl-1 expression 48 h after the treatment of Caco-2 cells with SU9516 and combination of ABT-737 with SU9516. The time course of decreased Mcl-1 expression correlated well with the MTT experiments that also showed positive effect of SU9516 on sensitivity to ABT-737 after the treatment of the cells with ABT-737 in combination with SU9516 for 48 h and more.

Our results are also in agreement with study documented that CDK2 inhibition by dinaciclib resulted in downregulation of Mcl-1 and the combination of dinaciclib with ABT-737 resulted in robust synergistic cell death in leukemic cells and primary chronic lymphocytic leukaemia patient samples (Choudhary et al. 2015). Treatment of HT29 with SU9516 was not associated with decrease of Mcl-1 expression that provides rational explanation about inefficiency of SU9516 to increase sensitivity of HT29 cells to ABT-737. Regulation of expression of Mcl-1 in cancer cells is very complex and depends on transcriptional, post-transcriptional and post-translational control (see for review Juin et al. 2014). It has been documented that CDK2-dependent phosphorylation of Mcl-1 resulted in increased Mcl-1 stability (Thr92, and Thr163) and BIM binding (Ser64) and CDK2 inhibition resulted in downregulation of Mcl-1 and BIM release from Mcl-1 in ABT-737 resistant cells (Choudhary et al. 2015). Mcl-1 can also be downregulated after the treatment with ABT-737 *via* caspase-3-mediated cleavage (Ryu et al. 2014). Additional effect of SU9516 to ABT-737 can be explained by the fact that inhibition of Mcl-1 phosphorylation is also associated with release of Mcl-1 from the binding to BIM that is pro-apoptotic BH3-only protein with great potential to induce ABT-737-mediated mitochondrial apoptosis (Del Gaizo Moore et al. 2007; Zhang et al. 2008b). Thus, our results related to Caco-2 cells are consistent with a view that phosphorylation of Mcl-1 is the major determinant of ABT-737 resistance in MCl-1 expressing cells (Mazumder et al. 2012). We can only speculate about the fact that Mcl-1 was not downregulated in HT29 cells after the treatment with SU9516. Interestingly, multikinase inhibitor BAY 43-9006 that downregulates Mcl-1 through inhibition of translation (Rahmani et al. 2005) or proteasomal degradation (Yu et al. 2005) was shown to downregulate Mcl-1 also in HT29 cells (Yu et al. 2005). Unlike ABT-737, pan-Bcl-2 inhibitor Obatoclax that binds to Bcl-2, Bcl-X$_L$, Bcl-w and Mcl-1 (Konopleva et al. 2008) was shown to be a potent late stage autophagy inhibitor in HT29 cells, causing cell growth arrest and cell death (Koehler et al. 2015).

In conclusion, our findings provide a rationale for clinical use of Bcl-2 family inhibitors in combination with CDK2 inhibitors for treatment of Mcl-1-dependent colorectal tumours associated with expression of Bcl-2, Bcl-X$_L$ and Bcl-w proteins. In addition, we have documented potential of CDK2 inhibitors for treatment of tumours expressing R273H mutant p53.

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