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Gene Expression Profile of Colon Cancer Cell Lines Treated with SN-38

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Introduction

Colorectal cancer (CRC) is one of the most common cancer forms worldwide, and the second leading cause of cancer deaths in the USA [1]. The incidence is relevantly high in Western countries, as there is an increased risk among those who have high dietary intake of red meat and low-fibre food, are obese and exercise rarely. Even though the incidence in Sweden has increased during the past 40 years, the mortality rate has remained constant due to early diagnosis, improvement in surgical skills and more efficient anti-cancer drugs. Almost 50% of all CRC patients will subsequently develop metastasis, which is the leading cause of CRC-related deaths. Metastatic CRC is treated with surgery whenever possible; however, systemic treatment is the main focus [2]. Many treatments are ineffective with a high frequency of chemo-resistance. It is therefore of great importance to find novel biological markers for treatment of metastatic CRC, which will also improve control of local recurrence that eventually leads to metastatic disease.

Irinotecan is a topoisomerase 1 (topo-1) inhibitor given to patients with refractory metastatic colorectal tumours and has been proved to significantly improve the overall survival of patients resistant to 5-fluorouracil treatment [3, 4]. The drug has been used clinically for several years, although studies are on-going in order to improve the regimen [5–7]. Irinotecan is converted by carboxylesterase to the active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38), which is 100- to 1,000-fold more active compared to the pro-drug [8, 9]. The cytotoxicity derives from the binding between SN-38 and the DNA-associated nuclear enzyme topo-1 which leads to inhibition of topo-1 and, as a consequence, single stranded (ss) DNA breaks. This mechanism becomes irreversible, leading to double stranded (ds) DNA breaks over time and subsequently to cell death and to tumour regression [10]. Irinotecan has been proven to exert cytotoxicity in a wide range of solid tumours including breast, colon, prostate and lung [11].

The formation of dsDNA breaks disrupts the cell cycle and triggers G2/M arrest. The status of cell cycle regulator p53 is of significance for the response of cell death. Wild-type p53 leads to long-term cell cycle arrest, i.e. cellular senescence, while mutated p53 gives premature mitosis, mitotic catastrophe and apoptosis.

In our previous study, the colon cancer cell lines KM12C, KM12SM and KM12l4a were treated with the topo-1 inhibitor SN-38. The results showed that the SN-38 treatment induced not only cell cycle arrest, but also apoptosis. Protein expression studies determined that the pathways including apoptosis-associated proteins like Bax and survivin were affected by the drug treatment [12]. In the survey to identify potential biological markers for treatment, it is of interest to further investigate the biological effect of SN-38 on cell lines. In the present study, the gene expression profiles of the cell lines treated with SN-38 were examined by microarray analysis.

Materials and Methods

Materials and Drug Treatments
KM12C, KM12SM and KM12L4a human colon carcinoma cell lines were kindly provided by Prof. I.J. Fidler (M.D. Anderson Cancer Center, Houston, Tex., USA). The parental cell line KM12C was originally established from a colon carcinoma Dukes' B2. Cells from this cell line were repeatedly injected into the cecum and spleen in athymic mice, to form 2 new cell lines KM12L4a and KM12L4a, both with high metastatic potential.

The cell lines were maintained in Eagle's MEM medium supplemented with 10% heat-inactivated foetal bovine serum, sodium pyruvate, vitamins and a cocktail of penicillin and streptomycin at 37 °C in 5% carbon dioxide (Gibco; Invitrogen, Paisley, UK). Cells growing exponentially were harvested when a confluency of 80% was achieved.

The prodrug irinotecan is converted by carboxylesterases to its active metabolite SN-38. We used the metabolite SN-38 (Aventis Pharma, Paris, France) for drug treatment and it was diluted to a stock solution of 50 mg/ml in dimethyl sulfoxide (DMSO). The stock solution was kept in −20 °C and diluted in medium to appropriate concentrations before use. For all experiments, cells were trypsinated, counted, plated in duplicates in 10-cm Petri dishes (Corning, Oneonta, N.Y., USA) with a seeding density of 0.33×10^6 cells/cm^2 and then incubated for 24 h to allow the cells to adhere. After 24 h, cells were treated with SN-38 (2.5 ug/ml) and harvested after 24 and 48 h, respectively. This protocol was originally designed in previous study by Wallin et al. [12] and in this paper the cellular phenotypes of the cell lines are more clearly depicted. Treated cells and controls were washed 3x in ice-cold PBS and then scraped into 5 ml of Trizol _ (Invitrogen, Carlsbad, Calif. USA). The cell lysates were kept in −70 °C until RNA was isolated. Untreated cells incubated with a DMSO concentration in the medium equivalent to the concentration SN-38 was diluted in, were used as controls for all experiments (referred to as 'untreated controls' in the text).

Preparation of RNA and Microarray
The RNA isolation were performed using Trizol (Invitrogen) followed by the RNeasy Midi kit (Qiagen, Valencia, Calif., USA), according to the manufacturers' manuals. The reference RNA used was the Universal Human Reference RNA (Stratagene, La Jolla, Calif., USA). Both RNA samples and reference were transcribed into cDNA and labelled with Cy3 and Cy5 using the CyScribe TM cDNA post-labelling kit (Amersham Biosciences, Amersham, UK). The RNA were annealed with Anchored oligo(dT) primer and an extension reaction was performed using CyScript TM reverse transcriptase. When synthesised, amino allyldUTP was incorporated into the cDNA. The remaining intact RNA was degraded by NaOH and the amino allyl-modified firststrand cDNA was purified using EtOH precipitation. In order to get a labelled cDNA probe, the purified amino allyl-modified cDNA was coupled with an excess of reactive CyDye TM NHS esters (Amersham Bioscences). Samples (treated cells and untreated controls) were coupled to Cy3 and reference cDNA to Cy5, then purified with the Cyscribe GFX purification kit to maximise the signal to noise ratio. The samples were pooled together and blocking reagents, Poly d(A) (Amersham Biosciences), Yeast tRNA (Sigma) and Human Cot-1 DNA (Invitrogen Life Technologies) were added as well before it was vacuum-dried to a pellet. Hybridisation was performed manually using the Pronto! universal hybridization kit (Corning Life Sciences, N.Y., USA), according to the manufacturer's instructions. The vacuum-dried pellet was resuspended in the Pronto! hybridization solution and applied to the slides. The slides were washed with the enclosed washing solutions and sealed in a Corning hybridization chamber at 42° for 18 h.

The human 70-mer oligonucleotide array consisted of ~27,000 spots with oligonucleotides supplied by the Operon V2 27k clones. The slides were manufactured at the Swegene DNA Microarray Resource Center, Department of Oncology, Lund University, Sweden.

Image and Data Analysis
All slides were scanned using an Agilent DNA microarray scanner (Agilent technologies, Palo Alto, Calif., USA) at 10 um resolution, setting the PMT (photo multiplier tube) in both

channels (red at 635 nm and green at 532 nm) to 100%. The images were analysed using GenePix Pro 4.1.1.4 (Axon Instruments, Union City, Calif., USA) which automatically aligned grids, associated spots with their reporter IDs and gene names and flagged spots 'found' or 'not found' depending of their quality. The automatic gridding and flagging was manually verified to eliminate artefacts and bad spots from subsequent analyses. GenePix Pro was then used to extract foreground and background pixel intensities for each spot into a GenePix result file which was then imported into the web-based BioArray Software Environment (BASE; http://base.thep.lu.se [13]) used for further data management and analyses.

Background corrections, filtering, transformations and statistical analyses were all performed uniformly on the array data within BASE. Background correction was performed by subtracting the median of the background pixel intensities from the median of the foreground or spot intensities in both channels for all spots. Preliminary quality filters were set to eliminate spots flagged 'not found' or 'bad', spots with diameters less than or equal to 55 um, spots with signal intensities less than or equal to zero and signal saturated spots in both channels. The background-corrected log-transformed intensity values were then normalised using the global LOWESS method where intensitydependant adjustments (LOWESS fits) were performed to compensate for dye bias [14]. Data from replicate spots on the same array were merged in a weighted fashion, as previously described [15]. The data were centred and transformed using the error-model in order to reduce the importance of poor-quality spots in later analysis steps [16]. Additional filters were applied to eliminate spots with multiple cluster hits, spots representing ESTs without any known function and spots with missing LocusLink identifiers. Furthermore, only spots with a minimum standard deviation (SD) of 0.2 and 65% presence across all hybridizations were used in the hierarchical cluster analysis. Unsupervised agglomerative hierarchical clustering based on the Pearson correlation distance metric was carried out using the freely available TMeV software (www.tm4.org/mev.html [17]) on the 5,915 spots that passed the various above-mentioned filter criteria. This was carried out in order to group together the control and treated cell lines harvested at different time points based on their similarities in gene expression profiles.

A supervised analysis was performed to identify the genes that differed in expression levels between the 6 treated cell lines and the 6 untreated control cell lines. The 5,915 genes were ranked on their Golub-scores or discriminative weight [18]. The relative expression ratios of each gene in the treated and untreated subclasses were used to calculate the mean (m) and the standard deviation (σ) for that particular gene across all the samples within each of the 2 subclasses. By using the mean and standard deviation calculated, each gene was assigned a discriminative score:

$G = (m_1 - m_0)/(\sigma_1 + \sigma_2)$

where m_1 and m_0 are the mean values and σ_1 and σ_0 are the standard deviations for subclasses 1 and 0, respectively. A high Golub-score suggests minor variation in gene expression within the subclass, but large variation between the subclasses. A random permutation test with 1,000 permutations was performed so as to assess the discriminating power of the score to differentiate the treated and untreated subclasses. For each score, the average number of genes in a permutation list above that score was divided by the number of genes in the true list to get the false-discovery rate. Moreover, functional analysis was performed on the up- and down-regulated genes using the freely available Expression Analysis Systemic Explorer (EASE) software [19] to identify functional groups, gene ontology categories and/or pathways that may be over-represented.

Results

KM12C, KM12SM and KM12L4a Treated with SN-38 in a Microarray Analysis
The 3 human colon cancer cell lines, KM12C, KM12SM and KM12L4a, were treated with topo-1 inhibitor SN-38 at a dose of 2.5 ug/ml for 24 and 48 h.

Oligonucleotide microarray slides comprising of ~27,000 spots were used for the expression analysis, and ~5,900 genes remained for the analysis after the application of various filters and transformation steps. The unsupervised hierarchical clustering performed on these 5,900 genes is shown in figure 1 a and clearly splits the treated samples from the untreated ones. Golub-score analysis identified 3,974 ($p = 0.05$) genes that differed in expression levels between the 6 treated and the 6 untreated cell lines. Figure 1 b shows the supervised hierarchical clustering based on the 3,974 ($p = 0.05$) discriminating genes. The 3 treated cell lines showed, regardless of time point (24 or 48 h), a majority of down-regulated genes when compared to the corresponding untreated cell lines. Of the 1,453 genes ($p = 0.001$), 1,036 were down-regulated, while only 417 were up-regulated.

The 6 untreated controls were analysed in order to unravel any difference between the 24 and 48 h. Out of 11,725 reporters with >65% presence, only 1,192 reporters had SD > 0.2 (i.e. variable expression). Of these 1,192, only 1 ($p = 0.001$), 32 ($p = 0.01$) and 128 ($p = 0.05$) reporters differed significantly between 24 h controls and 48 h controls. And only 44 reporters ($p = 0.05$) differed significantly between the primary control (KM12C) and the derived metastatic controls (KM12SM and KM12L4a). The 6 SN-38-treated cell lines were also analysed in the same way, giving the result that 3,234 reporters out of 11,744 with > 65% presence had SD > 0.2. Of these 3,234, none ($p = 0.001$), 40 ($p = 0.01$) and 172 ($p = 0.05$) differed significantly between the 24 h treated and 48 h treated cell lines. Only 13 genes out of the 172 ($p = 0.05$) overlapped with the 128 above-mentioned genes (distinguishing the 24 h controls from the 48 h controls).

The variation in gene expression among the treated samples compared to the untreated (3,234 variable genes vs. 1,192 in the case of the controls) for both 24 h treated and 48 h treated cell lines results in only 172 ($p = 0.05$) differentiating genes (almost as many as in the case of the untreated controls − 128). The 6 samples treated in 24 h (3 controls + 3 treated) and the 6 samples treated in 48 h (3 controls + 3 treated) were not analysed separately, due to no significant difference between the 24 and 48 h controls or between the 24 and 48 h treated samples. A considerable amount of overlap between the two gene lists (24 h control vs. 24 h treated and 48 h control vs. 48 h treated) would also be expected. Regarding all 12 samples (treated vs. controls), of 11,126 genes with presence in > 65% of the samples, 5,915 had SD > 0.2, indicating a strong signal differentiating the treated from the controls with 3,974 ($p = 0.05$), 2,978 ($p = 0.01$) and 1,453 ($p = 0.001$) significant genes.
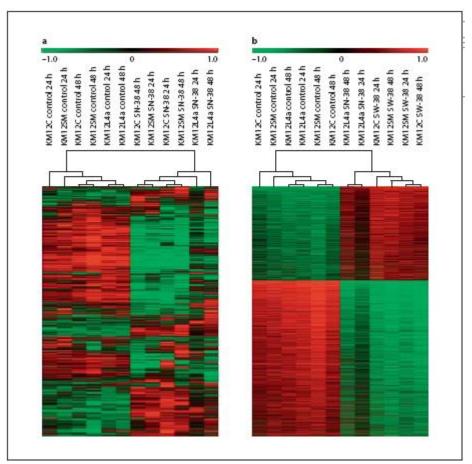
Fig. 1. *Clustering was performed using the TMeV software and the Pearson centred correlation distance metric.* **a** *Unsupervised clustering of the 12 samples based on all 5,915 genes that passed the quality, variation and presence filters.* **b** *Supervised clustering of the 12 cell lines based on the top 3,974 discriminating genes identified by the Golub-score analysis distinguishing the 6 treated cell lines from the 6 untreated.*

In order to group the genes based on their functional classification, the EASE functional analysis tool was used. The data retrieved from the Golub-score analysis (cut-off value p ≤ 0.05), where genes were divided into up- and down-regulated ones, were further explored with EASE analysis which resulted in 5 main functional groups among the down-regulated genes (table 1): RNA synthesis/RNA metabolism/RNA processing/RNA modification/RNA splicing/RNA binding; protein synthesis/protein metabolism/chromosome/nuclear and cell biogenesis and organization; protein transport/nuclear and cell biogenesis and organization; cell cycle, and DNA metabolism/chromosome/nuclear biogenesis and organization. Up-regulated genes were restricted to 4 main functional groups (table 2): receptor activity/signal transduction/cell communication; development/differentiation/morphogenesis; immune response, and transporters/channels.

Top Scores of Down- and Up-Regulated Genes
Among the down-regulated genes, the Golub-score range for the top 10 down-regulated genes was from 5.34 *(IBTK)* to 6.63 *(DOK6)* (table 3). Of these 10 genes the majority of them were involved in cell proliferation, RNA processing and apoptosis.

Table 1. *Summary of the EASE functional analysis for the down-regulated genes (EASE score ≤0.05)*

System/ Gene category	Functional group	Genes n
Biochemical function		
RNA-binding protein	1	93
Cellular role		
RNA processing/modification	1	70
RNA splicing	1	32
GenMAPP pathway		
Hs_tRNA synthetases	1	14
GO biological process		
RNA metabolism	1	146
RNA processing	1	138
Metabolism	2	1,270
RNA splicing	1	47
Nucleobase/nucleoside/nucleotide and nucleic acid metabolism	1	617
mRNA metabolism	1	61
Intracellular protein transport	3	110
mRNA processing	1	55
RNA splicing via transesterification reactions	1	37
RNA splicing via transesterification reactions with bulged adenosine as nucleophile	1	37
Nuclear mRNA splicing via spliceosome	1	37
Intracellular transport	3	141
Protein transport	3	112
Protein metabolism	2	503
Cell cycle	4	192
rRNA metabolism	1	22
Ribosome biogenesis and assembly	2	27
Nuclear organization and biogenesis		62
Translation	2	64
Ribosome biogenesis	2	26
mRNA splicing	1	19
Mitotic cell cycle	4	108
Chromosome organization and biogenesis (sensu Eukarya)		60
DNA metabolism	5	139
Cell organization and biogenesis		169
Protein biosynthesis	2	137
Ubiquitin-dependent protein catabolism	2	38
Modification-dependent protein catabolism	2	38
M phase	4	53

System/ Gene category	Functional group	Genes n
GO cellular component		
Intracellular		1,476
Nucleus		678
Spliceosome complex	1	41
Ribonucleoprotein complex	2	127
Cytoplasm		906
Cell		1,848
GO molecular function		
RNA binding	1	151
Nucleic acid binding	1	616
ATPase activity		76
ATPase activity coupled		72
Hydrolase activity acting on acid anhydrides in phosphorus-containing anhydrides		80
Helicase activity		47
ATP-dependent helicase activity		42
Hydrolase activity acting on acid anhydrides		124
mRNA binding	1	36
Protein transporter activity	3	78
ATP binding		252
Purine nucleotide binding	1	304
Nucleotide binding	1	306
Pre-mRNA splicing factor activity	1	27
Adenyl nucleotide binding	1	253
Ligase activity		89
KEGG pathway		
Aminoacyl-tRNA biosynthesis – *Homo sapiens*	1	15
Molecular localization		
RNA-associated	1	79
Organismal role		
General cellular role		26
Subcellular localization		
Nuclear		232

Functional groups: 1 = RNA synthesis, metabolism, processing, modification, splicing and binding; 2 = protein synthesis, protein metabolism, chromosome, nuclear and cell biogenesis and organization; 3 = protein transport, nuclear & cell biogenesis & organization; 4 = cell cycle; 5 = DNA metabolism, chromosome and nuclear biogenesis and organization.

All up-regulated genes were listed in the correspondence to their Golub-score. The 10 genes with the highest Golub-scores in the list were selected for further evaluation (table 4). The gene *SGK (SGK-1)* was found to be of highest significance, with a Golub-score of 6.48, and *TXNIP* had the lowest score (4.22). Among the highly expressed genes, some were involved in apoptosis, e.g. *SGK, RHOB* and *BAX* . Another major groups consists of genes involved in transcription *(SERTAD1* and *ZNF26)* , and in development and differentiation *(HMX1* and *ADM)* .

Discussion

SN-38, as an active metabolite of topo-1 inhibitor irinotecan, binds to topo-1 and stabilizes thereby the cleavable complexes. This leads to ssDNA breaks that are subsequently converted into dsDNA breaks. Thus, cell cycle arrest is induced due to the impaired DNA processing and, as a consequence, cell death is executed.

In our previous study, we treated colon cancer cell lines KM12C, KM12SM and KM12l4a with SN-38, and found that S phase and G2 arrest were induced together with increased apoptotic cell death, increased Bax protein expression and decreased topo-I protein

expression [12]. In the present study, we further examined the gene expression profile of the same cell lines treated with SN-38. Analyses of the functional groups of the genes showed that apoptosis-associated genes were highly affected by SN-38 in both down- and up-regulated genes. The majority of the genes (1,036) were down-regulated and only 417 genes were up-regulated.

Table 2. *Summary of the EASE functional analysis for the upregulated genes (EASE score ≤0.05)*

System/ Gene category	Func- tional group	Genes n
GO biological process		
G-protein-coupled receptor protein signalling pathway	1	73
Cell-to-cell signaling	1	61
Response to external stimulus	3	137
Organogenesis	2	115
Cell surface receptor-linked signal transduction	1	119
Morphogenesis	2	125
Development	2	202
Defence response	3	78
Cell communication	1	297
Immune response	3	69
Response to biotic stimulus	3	84
Neurogenesis	2	57
Response to pests/pathogens/parasites	3	46
Calcium ion transport	4	15
Response to abiotic stimulus	3	58
Ion transport	4	62
Di-/trivalent inorganic cation transport	4	20
GO cellular component		
Plasma membrane	1	186
Extracellular		118
Integral to plasma membrane	1	123
Voltage-gated calcium channel complex	4	9
GO molecular function		
Signal transducer activity	1	238
Receptor binding	1	70
G-protein-coupled receptor activity	1	48
Rhodopsin-like receptor activity	1	38
Channel/pore class transporter activity	4	48
α-type channel activity	4	45
Receptor activity	1	143
Ion channel activity	4	41
Calcium channel activity	4	16
Cation channel activity	4	32
Voltage-gated calcium channel activity	4	11
Transmembrane receptor activity	1	83
Biochemical function		
Inhibitor or repressor		57

Functional groups: 1 = receptor activity/signal transduction/cell communication; 2 = development and differentiation; 3 = immune response; 4 = transporters/channels.

Table 3. *Top 10 down-regulated genes of KM12C, KM12SM and KM12l4a treated with SN-38 in 24 and 48 h*

Rank	Reporter ID	LocusLink ID	Gene name	Gene symbol	Golub-score
1	H200010904	220164	DOK6: docking protein 5-like	DOK6	6.63
2	H200001879	91461	LOC91461: hypothetical protein LOC91461	LOC91461	6.59
3	H200020410	10521	DDX17: DEAD box polypeptide 17 isoform 2	DDX17	6.56
4	H200015842	80746	TSEN2: tRNA splicing endonuclease 2 homolog	TSEN2	6.51
5	H200006542	8439	NSMAF: neutral sphingomyelinase (N-SMase) activation	NSMAF	5.95
6	H200000904	57017	COQ9: coenzyme Q9 homolog	COQ9	5.95
7	H200008842	10152	ABI2: abl interactor 2	ABI2	5.85
8	H200008241	5074	PAWR: PRKC, apoptosis, WT1, regulator	PAWR	5.63
9	H200016133	4357	MPST: 3-mercaptopyruvate sulphurtransferase	MPST	5.47
10	H200001426	25998	IBTK: inhibitor of Bruton's tyrosine kinase	IBTK	5.34

Among the down-regulated genes, *DOK6* was on the top of the gene list, a gene that interacts with proto-oncogene *RET* . In thyroid carcinoma xenografts, inhibition of RET significantly increased the apoptotic response. *IBtk* was another down-regulated gene, and it functions as an inhibitor to Btk and subsequently as an inhibitor of NFκB-driven transcription [20]. Over-expression of the proto-oncogene K-ras is frequent in CRC and associated with, for example, a high proliferative state [21]. After SN-38 treatment, *K-ras* expression was down-regulated, although with low significance (Golub-score < 3). Moreover, an under-expression of *survivin (BIRC5)* was detected, also with a Golub-score < 3, indicating low significance. Survivin, as a member of the inhibitor of apoptosis (IAP) family, blocks the apoptotic process by inhibiting primarily caspases 3 and 7 and is proposed as a target for drug therapies due to its limited expression in tumour cells [22] .

Table 4. Top 10 up-regulated genes of KM12C, KM12SM and KM12l4a treated with SN-38 in 24 and 48 h

Rank	Reporter ID	LocusLink ID	Gene name	Gene symbol	Golub-score
1	H200009671	6446	SGK: serum/glucocorticoid-regulated kinase	SGK	6.48
2	H200013928	7262	PHLDA2: pleckstrin homology-like domain family A member	PHLDA2	6.05
3	H200006251	8870	IER3: immediate early response 3	IER3	5.76
4	H200014980	388	RHOB: ras homolog gene family, member B	RHOB	5.73
5	H200011292	3166	HMX1: homeo box (H6 family) 1	HMX1	5.02
6	H200006872	54461	FBXW5: F-box and WD repeat domain containing 5	FBXW5	4.74
7	H200006120	1490	CTGF: connective tissue growth factor	CTGF	4.64
8	H200004691	29950	SERTAD1: SERTA domain containing 1	SERTAD1	4.56
9	H200005992	6903	TBCC: β-tubulin cofactor C	TBCC	4.43
10	H200008354	10628	TXNIP: thioredoxin interacting protein	TXNIP	4.22

Of the up-regulated genes based on the Golub-score, the top 4 genes *(SGK , PHLDA2 , IER3 , and RhoB)* are all implicated in apoptosis. For example, *RhoB* , a member of the RAS superfamily and which functions as a tumour suppressor [23], was highly ranked. Also ranked highly was *IER3* , which through the inhibitory effect of NFκB [24] also plays the role of tumour suppressor. Further pro-apoptotic *Bax* was also found to be up-regulated, as was *IκBB (NFκBIB)* , an inhibtor to NFκB [25] . Up-regulation of *Bax* after SN-38 treatment has been previously demonstrated [26, 27], and over-expression of protein Bax has also been favourable in the way it enhances the induction of apoptosis [28].

Some genes were down- or up-regulated after the SN-38 treatment, in an opposite manner to that expected. For example, *WT1* (*PAWR*) was down-regulated, although it functions as a tumour suppressor in the way it represses transcription, along with the function of an apoptosis inducer via Bak [29].

Taken together, the genes involved in cell proliferation and apoptosis seem to be affected by SN-38 in a positive manner, i.e. promoting cell cytotoxicity. The status of tumour suppressor p53 is widely implicated in the success of chemotherapy [30], mainly through its role in apoptosis. The 3 cell lines in this study, KM12C, KM12SM and KM12L4a, are p53 mutated [31]. It is postulated that 2 different events could occur after SN-38 treatment depending on the p53 status. For p53 wild-type cells, cellular senescence is induced via S/G2 arrest that subsequently becomes permanent, compared to p53 mutated cells which will suffer from mitotic catastrophe and apoptosis [32, 33]. This corresponds with the results of altered expressions of apoptosis-associated genes here described.

It is widely discussed whether the expression of *topo-1* is altered following SN-38 treatment. In this study no such evidence of change in RNA expression after SN-38 treatment could be found and this was consistent with other findings [8, 27, 33]. The response to irinotecan is perhaps independent of *topo-1* expression [34]. Although in our previous study, topo-1 protein expression was found to be down-regulated following SN-38 treatment [12] . Changes in RNA expression do not necessarily have to have a direct impact on the protein expression. It could be possible that the deviant expression derives from posttranscriptional or translational alterations.

It is noteworthy that the cell lines display very similar gene expression profiles. From the parental cell line KM12C, two metastatic cell lines, KM12SM and KM12 L4a, were established. Even though the ability to form metastasis may vary between the cell lines, the response to the drug SN-38 is similar, indicating a nonsignificant influence on the metastasis-related factors. In our earlier study, different protein expression levels were detected depending of the metastatic potential [12]. However, in this current study all samples were analysed together for the gene expression profile and not as individual samples. In other words, the exact expression of each cell line could not be detected. Therefore, it is required that the expression of certain genes are analysed for each specific cell line.

In conclusion, the present results indicate that the expression of the genes involved in cell proliferation and apoptosis was affected by SN-38, based on their RNA expression. The impact of certain genes on CRC development needs to be further investigated; however, these results could serve as a basis for further studies in order to find targets for irinotecan treatment.

Acknowledgments

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