Association of a single nucleotide polymorphic variation in the human chromosome 19q13.3 with drug responses in the NCI60 cell lines

Kari K. Nissen[a], Ulla Vogel[b] and Bjørn A. Nexø[a]

We studied the importance of certain polymorphisms on human chromosome 19q13.3 for drug sensitivity in human tumor cell cultures. NCI60 is a panel of 60 established tumor-derived cell lines, which have been tested for their sensitivity to tens of thousands of different drugs. Here we investigate the correlations between the responses of the NCI60 cells to different anticancer drugs and their respective alleles of five DNA polymorphisms located in a cancer-related chromosomal area. One polymorphism, located in the 5′ noncoding region of the gene *ASE-1*, alias *CD3EAP*, proved to be associated with drug sensitivity ($P=0.025$). The same polymorphism has previously been associated with treatment response of multiple myeloma after bone marrow ablation. The polymorphism *ASE-1*-e1 was of importance for the drug response in the human cancer cell lines investigated and could eventually become important for individualized drug treatment in humans. *Anti-Cancer Drugs* 20:174–178 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2009, **20**:174–178

Keywords: *ASE-1*, *CD3EAP*, human chromosome 19q13.3, NCI60, single nucleotide polymorphisms

[a]Department of Human Genetics, University of Aarhus, Aarhus C and [b]National Food Institute, Søborg, Denmark

Correspondence to Bjørn A. Nexø, PhD, Department of Human Genetics, Bartholin Building, Wilhelm Meyers Allé, University of Aarhus, DK-8000 Aarhus C, Denmark
Tel: +45 89421686; fax: +45 86123173; e-mail: nexo@humgen.au.dk

Received 16 September 2008 **Revised form accepted** 17 November 2008

Introduction

The NCI60 human tumor cell line anticancer drug screen is a large-scale project commenced in late 1980s by the US National Cancer Institute (NCI). The purpose of this project was to expand the knowledge of anticancer drugs, and it was instigated because of the inadequacy of the previous tools based on murine models. The basis of NCI60 is a panel of 60 established tumor-derived cell lines from nine different tissues, which have been tested for their sensitivity to tens of thousands of mainly anticancer drugs. The establishment of this pool of cells and the related information has provided a backdrop for a wide range of discoveries in the field of anticancer research [1].

The chromosomal region 19q13.3 contains several genes important in DNA repair, more specifically nucleotide excision repair, and apoptosis mechanisms, namely the genes *XPD*, *ERCC1* and *RAI* (alias *PPP1R13L*, alias *iASPP*). In addition, the gene *ASE-1* (alias *CD3EAP*), with a less well-defined function, is found in this area. The protein product of this gene localizes in the fibrillar centers of nucleolus and might be important in rRNA transcription [2].

As DNA repair and apoptotic response are important factors in relation to cancer development and in chemotherapeutic treatment, exploiting extensive DNA damage, variations in genes involved in these mechanisms could play an important role in cancer development, progress, and treatment success. Consistent with this, extensive studies have shown polymorphisms in the 19q-13.2-3 region to be associated with certain forms of cancer, especially basal cell carcinoma, breast cancer and lung cancer [3–11]. Furthermore, Vangsted *et al.* [12] showed that some polymorphisms in this area influence the treatment outcome after chemotherapy and autologous stem cell transplantation in multiple myeloma patients.

In order to gain evidence of a more large-scale effect of this chromosomal area on the response toward chemotherapeutic drugs, we investigated the association between the sensitivity of the NCI60 cell line panel toward a judicious selection of the huge number of drugs tested and their respective genetic variants of different polymorphisms in the 19q13.2-3 region.

Methods

DNA from 59 out of 60 cancer cell lines in NCI60 program (omitting the breast cancer cell line MDN, which is no longer available) was generously provided by the NCI, after extraction with Qiagen Maxi Kit (Qiagen, Inc., Valencia, California) and in concentration close to 50 ng/μl.

Single nucleotide polymorphism (SNP) genotypings were performed on a LightCycler 480 (Roche, Hvidovre,

0959-4973 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e3283229ae3

Table 1 Primers and probes for the assays

Polymorphism	Primers, 100 pmol/μl	Probes, 20 pmol/μl
ERCC1-e4	F: 5′-TTCCTGAAGTCTGGGGTGG-3′	An: 5′-LCRed640-TGGCGACGTAATTCCCGACTATGTGCTG-P3′
rs11615	R: 5′-GACCACAGGACACGCAGA-3′	Se: 5′-CGCAA**C**GTGCCCTGGGAAT-X3′
ERCC1-3′	F: 5′-GGACAGATGGCAATGATGG-3′	An: 5′-CCACCACCTCTGTCTCTGGCT-FL3′
rs2336219	R: 5′-TCTTCTTCTTGGTGGATGTGG-3′	Se: 5′-LCRed640-CACTGTGGCAT**C**TTGCTG-P3′
RAI-i8	F: 5′-TGGCTAACACGGTGAAACC-3′	An: 5′-GGGAGGCGGAGCTTGCAGTGA-X 3
rs1970764	R: 5′-GGAATCCAAAGATTCTATGATGG-3′	Se: 5′-LCRed640-CTGAGATCGC**A**CCACTGCAC-P3′
ASE-1-e1	F: 5′-GGTTTTCTGCTCTGCACACG-3′	An: 5′-TCTGCAACCTGGTGCGAGCAGC-X3′
rs967591	R: 5′-CCTTTCTCCTTCCACCAACG-3′	Se: 5′-LCRed640-CGGGCTACAGGGTT**A**CCTGAG-P3′
RAI-3′-d1	F: 5′-AAAAAAATAGCCGAGCATGG-3′	
rs7255792	R: 5′-6FAM-TTTGGACTGGGTAAGAATTTCC-3′	

Denmark), a final reaction volume of 10 μl containing 5.72 μl H_2O, 1 μl titanium buffer, 0.05 μl of each primer, 0.1 μl of each probe, 0.13 μl dNTP (10 mM), 0.25 μl Titanium Taq DNA polymerase, and 2.5 μl DNA template (Table 1).

All programs were initiated with 10 min preincubation at 95°C to activate the polymerase, thereafter 45 cycles of amplification optimized for each SNP; *ASE-1*-e1 (rs967591): 5 s 95°C – 15 s 62°C – 12 s 72°C; *RAI*-i8 (rs1970764): 5 s 95°C – 10 s 60°C – 15 s 72°C; *ERCC1*-e4 (rs11615): 5 s 95°C – 15 s 61°C – 20 s 72°C; *ERCC1-3′* (rs2336219): 5 s 95°C – 15 s 60°C – 11 s 72°C. A melting curve program terminated the run with 1 min of 95°C, 1 min of 40°C, a temperature gradient to 75°C with 1 acquisition per °C and cooling to 40°C. All runs included negative controls and 20% of samples were repeated in random checks in independent runs with identical results.

The length of *RAI*-3′d1 polymorphism was analyzed on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with ROX size standard, after an initial PCR with fluorescein-labeled primers of 5 min 94°C, 30 cycles of 30 s 94°C – 30 s 62°C – 36 s 72°C and with a final annealing of 6 min 72°C.

Statistics

Data for $-\log_{10}$(GI50) (GI50=the drug concentration causing growth inhibition of 50% of the cell population) for the 59 cell lines were extracted from the NCI database(13). The dataset was reduced to 3634 drugs using two consecutive criteria: first, all drugs that had been tested only once were omitted. Next, the average variance of the $-\log_{10}$(GI50) within all cells was compared with the variance of $-\log_{10}$(GI50) between all cells for each drug. If the between-cell variance was less than 1.5 times the within-cell variance the drug was left out. This corresponded typically to a demand that an overall difference in the effect of the individual drug on the 59 cell types was present at a 5% level of significance. It served to focus the analysis on the informative drug tests. Finally, each drug response was normalized over the 59 cells by using the mean cellular response and the between-cells standard deviation. This step served to make the cellular responses to different drugs comparable. All these calculations were made taking the number of tests made with each drug–cell combination into account.

For comparing the drug response of cells with different genotypes, the cell lines were sorted into two groups: those with the common homozygous genotype; and those with the rare homozygous genotype and the heterozygotes. This also corresponds to the combinations that were previously reported as cancer relevant [4,6]. We compared the normalized values for all drugs and cells in each of the two groups using a *t*-value as test statistic. This was done for each polymorphism by calculating the mean $-\log_{10}$(GI50) toward all drugs for each cell line, and compare these values in the two groups by means of *t*-tests. We also compared all normalized test values in the two genotype groups with each other and calculated a *t*-statistic. In this case, because of the large amount of structure in the data we chose not to use normal-distribution statistics. Instead, we evaluated the significance of the *t*-value using permutation tests. The reference distribution was calculated by performing 10 000–40 000 *t*-tests after randomized assignment of the genotypes.

Results

The polymorphisms investigated here were chosen based on previous studies and included four SNPs and a tandem-repeat length polymorphism. Three of the SNPs, *RAI*-i8 (previously *RAI*-i1), *ERCC1*-e4, and *ASE-1*-e1, have been shown to constitute a high-risk haplotype associated with cancer [4,5,13,14], and in some cases showing association with cancer individually. *ASE-1*-e1 has also been shown to associate with treatment outcome in chemotherapy of myeloma patients [12]. The same is true for *ERCC1-3′* (unpublished results). The in-del length polymorphism *RAI*-3′d1 consists of a five base sequence (ATTTT), repeated various numbers of times in different chromosomes. Owing to previous investigations of association, six repeats or fewer was defined as 'short' and seven repeats and more as 'long'. The homozygous short version has been associated with increased risk of developing breast cancer, especially in younger postmenopausal women [6].

Table 2 Suggestive associations from calculations with the COMPARE program

Chemical	Polymorphism	*P* value
NSC 91580, αβ-digoxin acetate	*RAI*-i8	0.000716
NSC 24817, A peltatin	*RAI*-3′d1	0.000684

Initially, an association of the DNA polymorphisms with responses to the individual drugs was sought for using the COMPARE program. Table 2 shows a few results, which may suggest associations; however, no *P* values were significant when the large number of tests was corrected for, using Bonferroni correction.

Instead we chose to compare the response of cells of each group of genotypes with all the selected chemicals. In this way we were not asking for an association with sensitivity to the individual drug, but looked for a general pattern in sensitivity to many drugs, which related to the polymorphisms. This was justified by the fact that cell proliferation, apoptosis, and to some extent DNA repair are general cellular responses, and could play a role regardless of the specific mechanisms of action of the drugs. The testing was carried out in two ways: first, a mean of the normalized drug responses was calculated for each cell line and the means for each genotype group were then compared in a *t*-test ($P=0.025$ for *ASE-1*-e1; one sided). Second, all normalized cell responses belonging to each genotype group were compared in a global *t*-test based on permutation ($P=0.027$ for *ASE-1*-e1; one sided) (Table 3). The use of one-sided tests was justified on the basis of the results in Ref. [12]. The two-sided *P* values, however, were significant ($P=0.050$) and almost significant ($P=0.053$), respectively.

The four other polymorphisms showed no significant association with drug response. Neither did the homozygous haplotype constituted by *ERCC1*-e4^{A}, *ASE-1*-e1^{G} and *RAI*-i8^{A}, which have previously been shown to entail increased risk of cancer development. As previous studies have revealed sex-specific associations for polymorphisms in this region and development of cancers and chemotherapeutic treatment outcome [6,12], tests were performed on the sex-sorted mean values; this did not increase the significance.

Table 3 Association of polymorphisms with overall normalized drug response

Polymorphism	Variant frequency (%)	Common mean[a]	Variant mean[b]	*P* value for means	*P* value for all drug tests (permutation)
ASE-1-e1	12	0.087	−0.167	0.025	0.027
RAI-i8	14.3	0.017	−0.042	0.336	0.610
RAI-3′d1	44	−0.072	0.048	0.174	0.772
ERCC1-3′	12.5	0.050	−0.081	0.155	0.235
ERCC1-e4	35.4	−0.016	0.014	0.408	0.513

[a]Mean of the normalized means for homozygotes of the common variant.
[b]Mean of the normalized means for heterozygotes and homozygotes with the rare variant.

Discussion

The field of cancer pharmacogenomics is given high expectations to tailor therapy to each individual patient's genotype and tumor characteristics, to target tumor cells with the most effective drug, and avoid undesirable damage to the normal cells dependent of genomic composition. A better understanding of the genetic influence on the response toward medical drugs could open a path toward individualized treatment.

In this study, we have seen a moderate but wide-ranging effect of a specific genetic variation, *ASE-1*-e1, in tumor cells of very different origin. This indicates that the diverse effect given by the polymorphism affects the cellular handling of various types of DNA damage, as different chemotherapeutic agents entail different forms of DNA damage.

Previous investigations have shown that genotyping using LightCycler 480 and ABI Prism 3100 (Applied Biosystems, Naerum, Denmark) in 99% of samples result in identical outcome as determined by Sequenom. This knowledge and the fact that all random checks (20% repeats) gave identical results signify no reason to doubt the genotyping results.

Exposition and information on each of the more than 3000 drugs included in this study is outside the scope of our work. A list of the 59 cell lines and their individual genotype, for each of the five polymorphisms investigated here, can be found in Table 4. This information, combined with the $-\log 10$ (GI50) information found on the NCI webpage [15], is sufficient for computing the reduced number of drugs by the procedure described earlier. Each drug has been assigned an National Service Center number by NCI. This number can be used to obtain further information on chemical structure, etc of the individual drug on the NCI webpage.

The polymorphism showing significant correlation with drug response is located in the 5′ noncoding region of exon 1 of the *ASE-1* gene, at position −21 from the first codon. For this reason, the polymorphism will not affect the amino acid sequence of the final protein, though it could affect transcription or translation efficiency. Alternatively, this variation has no effect on itself, but serves as a marker for a nearby causative variation because of linkage disequilibrium. As *ASE-1* (*CD3EAP*) is a relatively short gene and is positioned close to the surrounding genes *ERCC1* and *PPP1R13L* (*RAI*), it is possible that the causative variation is found in either of these. The analysis of 59 genomes is insufficient for an accurate mapping of the causative variant.

In our study, the allele *ASE-1*-e1^{G} was associated with high sensitivity toward drug treatment, as the mean

Table 4 Genotypes of the five polymorphisms for the 59 cell lines

	rs2336219	rs1970764	rs967591	rs11615	rs7255792
Cell name	*ERCC1*-3′	*RAI*-i8	*ASE*-e1	*ERCC1*-e4	*RAI*-3′d1
CCRF-CEM	AG	GG	GG	AG	LL
HL-60(TB)	GG	AA	GG	AG	SL
K-562	GG	AA	GG	AA	SS
MOLT-4	GG	AA	GG	AA	SS
RPMI-8226	GG	AA	GG	AG	LL
SR	GG	AA	GG	AG	SL
A549/ATCC	GG	AA	GG	AA	SS
EKVX	AA	AA	AA	GG	SS
HOP-62	GG	AA	GG	AA	SS
HOP-92	GG	AA	GG	GG	SS
NCI-H226	AG	AA	AG	AA	SS
NCI-H23	AA	AA	AA	GG	LL
NCI-H322M	GG	AA	GG	AA	LL
NCI-H460	AG	AA	GG	GG	LL
NCI-H522	AA	AA	AA	GG	LL
COLO 205	GG	AG	GG	AG	SL
HCC-2998	AG	AG	AG	AG	SL
HCT-116	GG	AA	GG	AA	SS
HCT-15	GG	AG	GG	AA	SL
HT29	GG	AA	GG	AG	SL
KM12	GG	AA	GG	AA	SS
SW-620	AA	AA	AA	GG	SS
SF-268	AG	AG	AG	AG	SL
SF-295	AG	AA	AG	GG	SS
SF-539	GG	AA	GG	AA	SS
SNB-19	AG	AA	AA	AG	SS
SNB-75	AG	AG	AG	GG	LL
U251	AG	AA	AG	AG	SS
LOX IMVI	GG	AA	GG	AA	LL
MALME-3M	GG	AA	AG	AG	SS
M14	GG	AA	GG	AG	SL
SK-MEL-2	GG	AA	GG	AA	SS
SK-MEL-28	AG	AG	AG	AG	LL
SK-MEL-5	GG	AG	GG	AA	SL
UACC-257	GG	AA	GG	AA	SS
UACC-62	AG	AA	AG	GG	SL
IGROV1	GG	AG	GG	GG	SL
OVCAR-3	AG	AA	AG	AG	LL
OVCAR-4	AG	AA	AG	AG	SL
OVCAR-5	GG	AA	GG	GG	LL
OVCAR-8	GG	AA	GG	AA	SS
SK-OV-3	AG	AA	AG	AG	LL
786-0	AG	AA	AG	AG	SL
A498	GG	AG	GG	AA	SL
ACHN	GG	AA	GG	AA	SS
CAKI-1	GG	AG	GG	AA	SL
RXF-393	GG	AA	GG	AA	SL
SN12C	GG	AA	GG	AA	SL
TK-10	GG	AA	GG	AG	SS
UO-31	AG	AG	AG	AG	SL
PC-3	GG	AA	GG	GG	LL
DU-145	AA	GG	AA	GG	LL
MCF7	GG	AG	GG	AA	SL
NCI/ADR-RES	GG	AA	GG	AA	SS
MDA-MB-231/ ATCC	AG	AG	AG	AG	SL
HS 578T	GG	AA	GG	AA	SS
MDA-MB-435	GG	AA	GG	AA	SS
BT-549	GG	GG	GG	AA	LL
T-47D	GG	AA	GG	GG	LL

L/S, long/short repeat sequence.

normalized $-\log_{10}$(GI50) for cell lines with genotype GG were 0.0874, whereas the mean $-\log_{10}$(GI50) for pooled AA and AG genotypes were −0.16714. This means that on average approximately two-fold higher drug concentration was needed to achieve 50% growth inhibition in cell lines containing an A allele.

The relationship between *ASE-1*-e1 on one side and cancer risk and prognosis on the other can be explained in several ways: First, cells containing GG genotype might have a lower performance in important factors such as apoptosis or repair in response to DNA damage, wherefore they have a higher risk of accumulating mutations in their DNA, resulting in a possible increased risk of cancer development. Conversely, the lower dose of compound needed to inhibit growth of cells containing GG genotype could mean that stalled cells escape the normal repair and apoptosis mechanisms activated in the context of cell division and therefore have a higher tendency to survive the DNA damage. This would be in accordance with the finding that myeloma patients with this genotype have a higher risk of treatment failure and poorer survival [12].

The results obtained in this study underscore the importance of the 19q13.3 chromosomal region for cancer. They indicate that the *ASE-1*-e1 polymorphism in the future may contribute to personalizing the treatment of cancer patients. In this way, the results encourage the correlation between SNP and chemosensitivity in primary human tumors in a more general perspective. Nevertheless, the results obtained in the NCI60 cells require further validation in independent sets of cancer cell lines.

Acknowledgements

The authors thank Dr Susan Holbeck, National Cancer Institute (DTP) USA, for her very helpful support of these studies. The authors appreciate statistical help from Dr Morten Frydenberg, Department of Biostatistics University of Aarhus. This work was supported by The NovoNordisk Foundation, The Danish Cancer Society, The Danish Cancer Research Foundation and Fabrikant Einar Willumsens Mindelegat. The funding agencies had no other part in this project. The authors have no financial or personal relationships with other people or organizations that could inappropriately influence this work.

References

1 Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. *Nat Rev Cancer* 2006; **6**:813–823.
2 Whitehead CM, Winkfein RJ, Fritzler MJ, Rattner JB. ASE-1: a novel protein of the fibrillar centres of the nucleolus and nucleolus organizer region of mitotic chromosomes. *Chromosoma* 1997; **106**:493–502.
3 Rockenbauer E, Bendixen MH, Bukowy Z, Yin J, Jacobsen NR, Hedayati M, *et al.* Association of chromosome 19q13.2-3 haplotypes with basal cell carcinoma: tentative delineation of an involved region using data for single nucleotide polymorphisms in two cohorts. *Carcinogenesis* 2002; **23**:1149–1153.
4 Nexø BA, Vogel U, Olsen A, Ketelsen T, Bukowy Z, Thomsen BL, *et al.* A specific haplotype of single nucleotide polymorphisms on chromosome 19q13.2-3 encompassing the gene RAI is indicative of post-menopausal breast cancer before age 55. *Carcinogenesis* 2003; **24**:899–904.
5 Vogel U, Laros I, Jacobsen NR, Thomsen BL, Bak H, Olsen A, *et al.* Two regions in chromosome 19q13.2-3 are associated with risk of lung cancer. *Mutat Res* 2004; **546**:65–74.

6 Nexø BA, Vogel U, Olsen A, Nyegaard M, Bukowy Z, Rockenbauer E, *et al*. Linkage disequilibrium mapping of a breast cancer susceptibility locus near *RAI/PPP1R13L/iASPP*. *BMS Med Genet* 2008; **9**:56.

7 Butkiewicz D, Rusin M, Enewold L, Shields PG, Chorazy M, Harris CC. Genetic polymorphisms in DNA repair genes and risk of lung cancer. *Carcinogenesis* 2001; **22**:593–597.

8 Caggana M, Kilgallen J, Conroy JM, Wiencke JK, Kelsey KT, Miike R, *et al*. Associations between ERCC2 polymorphisms and gliomas. *Cancer Epidemiol Biomarkers Prev* 2001; **10**:355–360.

9 Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, *et al*. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* 2001; **61**:1354–1357.

10 Sturgis EM, Zheng R, Li L, Castillo EJ, Eicher SA, Chen M, *et al*. XPD/ERCC2 polymorphisms and risk of head and neck cancer: a case-control analysis. *Carcinogenesis* 2000; **21**:2219–2223.

11 Tomescu D, Kavanagh G, Ha T, Campbell H, Melton DW. Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. *Carcinogenesis* 2001; **22**:403–408.

12 Vangsted A, Gimsing P, Klausen TW, Nexo BA, Wallin H, Andersen P, *et al*. Polymorphisms in the genes ERCC2, XRCC3 and CD3EAP influence treatment outcome in multiple myeloma patients undergoing autologous bone marrow transplantation. *Int J Cancer* 2007; **120**:1036–1045.

13 Vogel U, Hedayati M, Dybdahl M, Grossman L, Nexø BA. Polymorphisms of the DNA repair gene XPD: correlations with risk of basal cell carcinoma revisited. *Carcinogenesis* 2001; **22**:899–904.

14 Yin J, Rockenbauer E, Hedayati M, Jacobsen NR, Vogel U, Grossman L, *et al*. Multiple single nucleotide polymorphisms on human chromosome 19q13.2-3 associate with risk of Basal cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2002; **11**:1449–1453.

15 WEBSITE development therapeutics program NCI/NIH; 2008; *http://dtp.nci.nih.gov*