Lack of EGF receptor contributes to drug sensitivity of human germline cells

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Germline mutations have been associated with generation of various types of tumour. In this study, we investigated genetic alteration of germline tumours that affect the drug sensitivity of cells. Although all germline tumour cells we tested were hypersensitive to DNA-damaging drugs, no significant alteration was observed in their DNA repair activity or the expression of DNA repair proteins. In contrast, germline tumours expressed very low level of epidermal growth factor receptor (EGFR) compared to drug-resistant ovarian cancer cells. An immunohistochemical analysis indicated that most of the primary germline tumours we tested expressed very low level of EGFR. In accordance with this, overexpression of EGFR in germline tumour cells showed an increase in drug resistance, suggesting that a lack of EGFR, at least in part, contributes to the drug sensitivity of germline tumours.

Keywords: DNA damage; epidermal growth factor receptor; drug resistance; cisplatin; cancer; chemotherapy

Germline mutations are associated with generation of various tumours. Previous studies indicated that germline cells were hypersensitive to DNA-damaging agents (Cagnoli et al, 1998). Although the mechanisms of, drug resistance are still poorly understood, an increased rate of DNA adduct removal appears to be associated with drug resistance in various human cancers (Lai et al, 1988; Dinapoli et al, 1993; Johnson et al, 1994; Eastman and Schulte, 1998). Drug-resistant human tumours have been shown to express higher levels of nucleotide excision repair (NER) proteins such as XPA, XPB (Lai et al, 1988; Eastman and Schulte, 1998), ERCC1, and cockayne syndrome group B (CSB) (Lai et al, 1988).

Also, altered expression of genes involved in 6-alkyltransferase-mediated direct DNA repair (O-6-methylguanine DNA methyl transferase, MGMT) or base excision repair pathway also contributes to drug resistance of cancer cells (Chetsanga and Lindahl, 1979; Doetsch and Cunningham, 1990; Cohen et al, 1991; Demple and Harrison, 1994; Gill et al, 1996; Deutsch et al, 1997; Asagoshi et al, 2000; Bielas and Heddle, 2000; Dobson et al, 2000; Evans et al, 2000). Defects in mismatch repair (MMR) are associated with cisplatin resistance by contributing to increased replication bypass of cisplatin adducts and to a drug-tolerant phenotype (Hansen et al, 1998; Karahalil et al, 1998; Hansen and Kelley, 2000; Limp-Foster and Kelley, 2000; O’Neill, 2000). Therefore, loss of MMR proteins such as hMLH1 leads to resistance of tumour cells to a variety of DNA-damaging agents, including bifunctional alkylating and monofunctional methylating agents

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Received 2 August 2004; revised 27 October 2004; accepted 8 November 2004; published online 11 January 2005

In this study, we investigated the genetic alteration of germline tumours such as altered DNA repair activity and/or damage signalling pathways that affect the drug sensitivity of cells. We found no significant change in NER activity or expression of DNA repair proteins in drug-sensitive germline cells compared to the drug-resistant ovarian cancer cells. Instead, the expression of a membrane receptor tyrosine kinase, EGFR, correlated with the
cells drug resistance. Drug-resistant cancer cells exhibited elevated level of EGFR expression, while drug-sensitive germine cells showed a lower EGFR expression. Overexpression of the EGFR gene significantly enhanced the cells drug resistance, suggesting that EGFR may be one of the contributing factors that affect drug resistance of cancer cells.

MATERIALS AND METHODS

Cell lines, cell culture, and drug treatment: NT2/D1 cells were obtained from American Type Culture Collection (Rockville, MD, USA) and 833K and 64CP9 GCT cell lines were obtained from G Sledge (Indiana University School of Medicine, Indianapolis, IN, USA). JNK1 and/or JNK1/2 were obtained from either Santa Cruz Biotech., Santa Cruz, CA, USA. All germline and ovarian cells were maintained in MEM supplemented with 10% fetal bovine serum at 37°C in a CO2 incubator, while IOS80 was maintained in MEM and 199/MCDB 105 (1:1) supplemented with 10% fetal bovine serum and EGF (10 ng ml^{-1}).

Germ cell tumours (GCTs): Tissue sections of biopsy materials with disseminated GCTs were obtained from the Indiana University Medical Center, University Hospital, under an Indiana University IRB. JNK1 and/or JNK1/2 were obtained from a human teratocarcinoma (Hep3B) by a peritoneal deposit of a cytopenic carcinoma of the ovary (from G Mills, MD Anderson Cancer Center, Houston, TX, USA), and a normal ovarian epithelial cell (IOSE80) was obtained from JA Hurteau (Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL, USA). All germline and ovarian cells were maintained in MEM supplemented with 10% fetal bovine serum at 37°C in a CO2 incubator, while IOS80 was maintained in MEM and 199/MCDB 105 (1:1) supplemented with 10% fetal bovine serum and EGF (10 ng ml^{-1}).

Immunofluorescence microscopy: PA-1 cells were grown on cover slides, washed twice with PBS, fixed in –10°C methanol for 5 min, air dried, and washed three times again with PBS. Fixed cells were incubated with an anti-EGFR polyclonal antibody (Santa Cruz Biotech., Santa Cruz, CA, USA) or Pharmingen (San Diego, CA, USA). For Western blot, cell lysates were loaded onto a 6 or 10% SDS-PAGE, and following gel electrophoresis proteins were transferred to nitrocellulose membrane and immunoblotted with primary antibody followed by a peroxidase-conjugated secondary antibody (Amersham) and an enhanced chemiluminescence (Amersham) reaction prior to visualisation. An enhanced chemiluminescence (Amersham) reaction prior to visualisation. A Kodak-O-mat film.

Transfection and selection of stable cell lines: Cells were transfected with either pEGFR-GFP or pEGFP-N3 using Lipofect AMINE method (Life Technologies Inc.). Following antibiotic selection with G418 (600 μg ml^{-1}) Geneticin-Life Technologies, Carlsbad, CA, USA) or Pharmingen (San Diego, CA, USA). Cell survival assay: To examine drug resistance of cells, cells (1.0 × 10^5 cells well^{-1}) were plated in a 96-well plate and incubated for 24 h. Cells were treated with drugs and further incubated at 37°C and 5% CO2 for 72 h. After 72 h incubation, cell survival was measured using a colorimetric cell survival assay from Boehringer Mannheim (MTT Cell Proliferation Kit). Alternatively, clonogenic assay was used to measure the ability of cells to form colonies on 100 mm^2 tissue culture dishes following treatment with ionising radiation or cisplatin. Controls consisted of cells untreated with peptides or DNA-damaging agent, or with neither. Cells were continuously exposed for 5 days to the indicated concentrations of the peptide, and colonies were stained with crystal violet and then colonies greater than 50 cells were counted. Each point represents mean values ± s.e., each conducted with triplicate plates.

Immunohistochemistry: Tissue sections were visualised for EGFR expression using an anti-EGFR monoclonal antibody (Santa Cruz Biotech., Santa Cruz, CA, USA). The Dako Universal Staining system (Dako Corp., Carpinteria, CA, USA) was used to automate the immunostaining procedure (Roberts et al, 2001). Sections were treated with 3% H2O2 for 10 min and incubated with an anti-EGFR antibody (1:1000) for 25 min, the biotinylated goat anti-mouse antibody IgG secondary antibody for 10 min, streptavidin-
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monitored using MTT assay and the results are the averages of three independent assays.

A cells (Hey). Cells were treated for 72 h with various concentrations of cisplatin (Figure 1B and C).

RESULTS

Germline cells exhibit hypersensitivity to DNA-damaging drugs

To analyse drug resistance of germline cells, four established germline tumour cells (PA-1, NT2/D1, 833K, and 64CP9) were compared with a normal ovarian epithelial cell line (IOSE80) and drug-resistant ovarian cancer cells (Hey) derived from a peritoneal deposit of a cystadenocarcinoma of the ovary (Figure 1). The established ovarian cancer cells (Hey) showed a marked resistance to cisplatin treatment, while the germline tumour cells were remarkably sensitive to the drug treatment (Figure 1). All four germline tumour cells showed extreme sensitivity to cisplatin treatment (5 μM) with a survival rate of less than 10%, whereas 80% of Hey cells survived under the same conditions. Meanwhile, a primary epithelial ovarian cell (IOSE80) showed a medium level of cell survival following cisplatin treatment (Figure 1). Adriamycin is a DNA-intercalating agent that causes DNA strand break damage, while MMC mainly causes DNA damage by forming a DNA crosslink. Similar to the cisplatin treatment, germline tumour cells were highly sensitive to both MMC and adriamycin treatment (Figure 1B and C).

Drug sensitivity of germline cells correlates with the lack of EGFR expression

To better understand the hypersensitivity of germline tumour cells to DNA-damaging drug, we analysed expression of various proteins that are involved in the drug sensitivity of cells. No noticeable difference was observed between drug-sensitive germline tumour cells and a drug-resistant cell (Hey) in the expression of DNA repair proteins (PCNA, TFIIH, DNA-PKcs, and Ku70/80) (Figure 2). We noticed however some difference in the expression of DNA-PKcs (Figure 2), although this subtle difference was not consistently observed in multiple experiments (data not shown). Interestingly, a significant difference was observed in the expression of EGFR between germline tumour cells and ovarian cancer (Hey) cells, while the expression of JNK1 and JNK2 showed no difference between them (Figure 2).

Expression of EGFR enhances the drug resistance of germline cells

To further examine whether the lack (or low level) of EGFR expression in germline tumour cells (Figure 3A) contributes to their drug sensitivity, cells were transfected with plasmid DNA expressing either green fluorescence protein (GFP) or GFP-EGFR fusion protein and analysed for their effect on drug resistance of cells. After initial selection of cells expressing GFP or GFP-EGFR, protein expression and cellular localization were analysed by Western blot (Figure 3B) and by fluorescence microscopy (Figure 3C), respectively. Germline tumour cells harbouring pEGFR-GFP plasmid showed a high level of EGFR expression, which was comparable to that in drug-resistant ovarian cancer (Hey) cells (Figure 3B). Cells harbouring pEGFR-GFP not pEGFP-N3 showed EGF-dependent activation of JNK1, suggesting that GFP-EGFR fusion protein is functionally active (data not shown).

Germline cells transfected with pEGFR-GFP showed only a marginal increase in their cell survival following cisplatin treatment, while cells expressing GFP (pEGFP-N3) exhibited a slight decline in cell survival (Figure 4 and Table 1). When a stably transfected cell instead of transient system was examined for drug sensitivity, however, it not only showed a significant increase in EGFR expression, but also enhanced survival of germline tumour cells following cisplatin treatment (Figure 5 and Table 1). Although EGFR kinase is activated by EGF, we did not see a substantial increase in cell survival in the presence of EGF probably because EGFR can also be activated by cisplatin. The difference in cell survival between transiently transfected cells (Figure 4) vs stable transfectants (Figure 5) following drug treatment may be due to the lower transfection efficiency in transient system, where only 30% of cells expressed GFP-EGFR (data not shown). Together, our results suggest that (1) a lack (or lower level) of EGFR expression

Figure 1 Effect of various drugs on the survival of GCTs (PA-1, 833K, NT2/D1, and 64CP9), ovarian primary epithelial cells (IOSE 80), and ovarian cancer cells (Hey). Cells were treated for 72 h with various concentrations of cisplatin (A), MMC (B), and adriamycin (C). Percentage of surviving cells was monitored using MTT assay and the results are the averages of three independent assays.
in germline tumour cells contributes to their drug sensitivity and
(2) EGFR may play a positive role in protecting cells following
treatment of cells with DNA-damaging agent.

Lack (or lower level) of EGFR expression in primary
germline cells
To see whether lack or lower level of EGFR expression is a
common property of germline tumour cells, a number of primary
GCTs were selected and tested for EGFR expression. Among 61
GCTs tested, 35 showed undetectable level of EGFR expression,
while the remaining samples expressed very low level of EGFR
compared to a control ovarian cancer cells (Table 2), supporting
the observation with established cells (PA-1, NT2/D1, 833K, and
64CP9) that germline tumours express lower level of EGFR
(Figure 3). In fact, the probability of all 61 GCT samples having
EGFR expression no higher than $+\alpha$ is extremely low ($2 \times 10^{-25}$).

DISCUSSION
Alteration of DNA repair factors or damage response proteins has
been associated with drug resistance of cancer cells (Mohrenweiser
et al., 2003). For example, a tumour suppressor gene, p53, is a key
DNA damage mediator that plays a dual role following exposure to
cytotoxic treatment (Ferrera et al., 1999); it is involved in damage-
induced apoptosis, but also plays a role in cell cycle arrest and
DNA repair, cellular processes that can affect the sensitivity to
chemotherapeutic drug. However, a consensus on the role for DNA
repair genes in drug resistance of various cancer cells has not been
reached, mainly because the complicated nature of drug-induced
resistance with various tumours made it difficult to delineate a
single mechanism (such as DNA repair) that contributes to the
resistance.

Compared to drug-resistant ovarian cancer cells, germline
tumour cells showed a marked sensitivity following the treatment

Figure 2 Expression of various proteins in germline cells. Extracts
(100 µg) from various germline tumour cells (PA-1, 833K, NT2/D1, and
64CP9) and ovarian cells (Hey and IOSE-80) were analysed for the
expression of DNA repair factors or damage signalling proteins by Western
blot.

Figure 3 Whole-cell lysates (30 µg) from various germline tumour cells (PA-1, 833K, NT2/D1, and 64CP9) and Hey cells were examined for the
expression of EGFR by Western blot (A). In (B), PA-1 cell lines were stably transfected with either pEGFP-N3 vector or pEGFR-GFP, while 833K and 64CP9
cells were transiently transfected for 36 h with pEGFP-N3 or pEGFR-GFP (see ‘Materials and Methods’ for the details). Expression of EGFR-GFP was
monitored by Western blot. (C) shows the expression of GFP (left) or GFP-EGFR (right) in PA-1 cells that were stably transfected with pEGFP-N3 or
pEGFR-GFP, respectively. For immunofluorescence, cells were fixed and permeabilised briefly with methanol incubated with anti-EGFR antibody as
described under ‘Materials and Methods’.
with cisplatin, adriamycin, or MMC (Figure 1). Examination of the established cell lines as well as primary germcell tumours for genetic alteration of several key repair factors and damage signalling factors indicated that drug sensitivity of germline tumour cells (833K (A); 64CP9 (B)) following cisplatin treatment. Control cells were compared with those transiently transfected with either pEGFP-N3 vector or pEGFR-GFP and examined for their cell survival following cisplatin treatment. At 24 h after transfection, cells were exposed to the indicated amount of cisplatin for 72 h. The percentage of surviving cells was monitored by MTT assay.

**Figure 4** Transient expression of EGFR enhances the survival of two germline tumour cells (833K (A); 64CP9 (B)) following cisplatin treatment. Control cells were compared with those transiently transfected with either pEGFP-N3 vector or pEGFR-GFP and examined for their cell survival following cisplatin treatment. At 24 h after transfection, cells were exposed to the indicated amount of cisplatin for 72 h. The percentage of surviving cells was monitored by MTT assay.

**Table 1** Effect of EGFR expression on cisplatin resistance of germline tumour cells (833 K) following cisplatin treatment

| Cisplatin (µM) | Mean cell survival rate (%) | P-value from t-test |
|---------------|-----------------------------|-------------------|
| (−EGF)        | pEGFP-N3 | pEGFR-GFP |                  |
| 0.1           | 81.25   | 95.5      | 0.006             |
| 0.5           | 69.25   | 78        | 0.008             |
| 1.0           | 49.25   | 57.25     | 0.002             |
| (+EGF)        | pEGFP-N3 | pEGFR-GFP |                  |
| 0.1           | 93.25   | 98        | 0.047             |
| 0.5           | 87.75   | 97.5      | 0.001             |
| 1.0           | 64.25   | 75.25     | 0.004             |

Cells expressing EGFR (pEGFR-GFP) were compared with control cells (pEGFP-N3) for cell survival following cisplatin treatment in the presence and absence of EGF (n = 4).

**Figure 5** Overexpression of EGFR markedly increased the survival of PA-1 cells following cisplatin treatment. Control cells (PA-1) were compared with those stably transfected with either pEGFP-N3 vector or pEGFR-GFP for their cell survival in the presence (A) and absence (B) of EGF following cisplatin treatment. At 24 h after the seeding, cells were exposed to the indicated amount of cisplatin for 7 days before counting colonies (>50 cells colony⁻¹). Each point is the mean value of triplicate experiments.
Table 2  Germ cell tumour (GCT) samples scored for EGFR expression by immunohistochemistry

| GCT#  | EGFR | GCT#  | EGFR | GCT#  | EGFR |
|-------|------|-------|------|-------|------|
| LSAB  | ++++ | GCT 1218 | + | GCT 1191 | – |
| GC control | + | GCT 1108-2 | + | GCT 1171 | – |
| GCT 1221 | + | GCT 1131-2 | – | GCT 1169 | – |
| GCT 1130-2 | – | GCT 1132 | – | GCT 1190 | – |
| GCT 1115 | – | GCT 1142 | + | GCT 1183 | – |
| GCT 1230-2 | – | GCT 1116 | – | GCT 1182 | – |
| GCT 1206 | – | GCT 1124 | – | GCT 1181 | + |
| GCT 1110 | – | GCT 1127 | + | GCT 1156 | – |
| GCT 1208 | + | GCT 1118 | – | GCT 1122 | + |
| GCT 1106 | – | GCT 1229 | + | GCT 1199 | – |
| GCT 1108 | + | GCT 1144 | + | GCT 1198 | + |
| GCT 1135 | – | GCT 1202 | + | GCT 1160 | + |
| GCT 1143-2 | – | GCT 1154 | + | GCT 1204 | – |
| GCT 1134 | – | GCT 1153 | – | GCT 1224 | + |
| GCT 1215 | – | GCT 1151 | + | GCT 1158 | + |
| GCT 1182-2 | – | GCT 1150 | – | – | – |
| GCT 1212 | + | GCT 1160-2 | – | – | – |
| GCT 1145-2 | – | GCT 1147 | + | – | – |
| GCT 1125 | + | GCT 1161-2 | – | – | – |
| GCT 1097-2 | + | GCT 2470-9 | – | – | – |
| GCT 1098 | – | GCT 1165 | – | – | – |
| GCT 1100 | + | GCT 1166 | – | – | – |
| GCT 1138 | + | GCT 1168 | – | – | – |
| GCT 1214 | – | GCT 1194 | – | – | – |

Two control cells (ovarian cancer cells (Hey, LSAB) and a normal germ cell, GC control) were included to compare the level of EGFR expression in GCT. ‘+’ represents no detectable EGFR expression, while the level of protein expression was determined by comparing with two control cells, GC control at the lowest level (+) and LSAB at the highest level (+++).

tumour cells may not be due to an alteration of repair factors or DNA repair activity (Figure 2). Instead, there was a good correlation between EGFR expression (or EGF-induced JNK activation) and drug resistance among ovarian and germline tumour cells. Low level of EGFR expression in germline tumour cells may be linked to their drug sensitivity and supports a positive role for EGFR in drug resistance of cancer. The latter may be explained by the fact that EGFR and its receptor activate the JNK signalling pathway that leads to the induction of genes involved in DNA repair and cellular redox (Adler et al, 1992; Foltz et al, 1998; Roulston et al, 1998).

Epidermal growth factor receptor is a 170 kDa transmembrane glycoprotein with tyrosine kinase activity. Although EGFR was shown to have no independent prognostic significance in advanced cancer (Baekelandt et al, 1999), the EGFR and HER2/neu were frequently overexpressed in malignant tumours. Recent microarray analysis revealed that amplification of EGFR gene was found in many tumours including ovarian cancer (Lei et al, 1999), glioblastoma (Hui et al, 2001), pancreatic cancer (Bruell et al, 2003; Schreiner et al, 2003), gastric cancer (Garcia et al, 2003), prostate cancer (Skacel et al, 2001), and lung adenocarcinoma and head/neck squamous cell carcinoma (Haedicke et al, 2003; Shintani et al, 2003), suggesting that overexpression of EGFR may be linked to the oncogenesis of various cancers. High level of EGFR expression also correlates with increased tumour resistance to radiation (Shintani et al, 2003), suggesting that EGFR may mediate radioresistance of cancer cells (Liang et al, 2003). Epidermal growth factor receptor is also a cellular receptor for human cytomegalovirus, a cancer-causing virus that causes severe and fatal disease in immune-compromised individuals (Wang et al, 2003).

Epidermal growth factor receptor-associated protein tyrosine kinase complexes also have vital antiapoptotic functions in human breast cancers (Modjtahedi et al, 1998; Witters et al, 1999) and the blockade of EGFR not only adversely affected cell growth, but also showed a sign of terminal differentiation and induces apoptosis in the human cancer cells (Modjtahedi et al, 1998). Similarly, drug-induced apoptosis in human breast cancer cells was abrogated by using EGFR antisense RNA (Dixit et al, 1997), suggesting that a critical level of EGFR signalling, which is amplified in some common cancers, may be necessary for DNA-damaging drug-mediated apoptosis in tumour cells and suggest an inhibitory effect of this pathway on the repair of cisplatin-damaged DNA. In fact, cancer cells expressing higher levels of EGFR were much more resistant to the growth inhibitory effect of DNA-damaging agents than were control cells (Dixit et al, 1997).

Various strategies have been developed to target EGFR and to deter cancer cell growth (Zhang et al, 2000; Bruell et al, 2003; Heimberger et al, 2003). For example, the treatment of cancer cells with EGFR tyrosine kinase inhibitor markedly potentiates the efficacy of many cytotoxic agents against several human cancer xenografts (She et al, 2003). The use of antisense oligonucleotides or monoclonal antibodies to EGFR also showed significant inhibition of cancer cell growth (Modjtahedi et al, 1998; Witters et al, 1999), while activation of EGFR family members suppresses the cytotoxic effects of TNF-alpha (Hoffmann et al, 1998).

Although mutations in proto-oncogenes (c-ret) as well as DNA MMR genes have been linked to germline tumours (van Puijenbroek et al, 1997; Leung et al, 2000), alteration of EGFR in germcell tumours has not been reported. This study showed that germline tumour cells not only exhibited lower EGFR expression but also were highly sensitive to DNA-damaging drugs, suggesting that the lack of EGFR expression contributes at least in part to the drug sensitivity of germline cells.

ACKNOWLEDGEMENTS

We thank Dr A Sorkin for providing us with pEGFP-N3 vector and pEGFP-EGFR, Dr R Bigsby for PA-1 cells, Dr G Mills for Hey and its derivative cells, and Dr J Hurteau for IOSE80 ovarian cell lines. This research was supported by grants from the US Army (DAMD17-00-1-0295) and the National Institute of Health (CA92111). S-J Park was supported by the NIH National Research Service Award (T32 DK07519).

REFERENCES

Adler V, Franklin C, Kraft A (1992) Phorbol ester stimulate the phosphorylation of c-Jun but not v-Jun: regulation by the N-terminal delta domain. Proc Natl Acad Sci USA 89: 5341 – 5345
Asagoshi K, Yamada T, Terato H, Ohyama Y, Monden Y, Arai T, Nishimura S, Aburatani H, Lindahl T, Ide H (2000) Distinct repair activities of human 7,8-dihydro-8-oxoguanine DNA glycosylase and formamidopyrimidine DNA glycosylase for formamidopyrimidine and 7,8-dihydro-8-oxoguanine. J Biol Chem 275: 4936 – 4964
Arteaga C (2003) Targeting HER1/EGFR: a molecular approach to cancer therapy. Semin Oncol 30: 3 – 14
Baekelandt M, Kristensen GB, Trope CG, Nesland JM, Holm R (1999) Epidermal growth factor receptor expression has no independent prognostic significance in advanced ovarian cancer. Anticancer Res 19: 4469 – 4474
Bielas JH, Heddle JA (2000) Proliferation is necessary for both repair and mutation in transgenic mouse cells. Proc Natl Acad Sci USA 97: 11391 – 11396
Bruell D, Stocker M, Huhn M, Redding N, Kupper M, Schumacher P, Paetz A, Bruns CJ, Haisma HJ, Fischer R, Finnern R, Barth S (2003) The recombinant anti-EGF receptor immunotoxin 425(scFv)-ETA’

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suppresses growth of a highly metastatic pancreatic carcinoma cell line. Int J Oncol 23: 1719 – 1726
Cagnoni M, Alama A, Barbieri F, Novelli F, Bruzzo C, Sparatore F (1998) Synthesis and biological activity of gold and tin compounds in ovarian cancer cells. Anticancer Drugs 9(7): 603 – 610
Chetsanga CJ, Lindahl T (1997) Release of 7-methylguanine residues whose imidazole rings have been opened from damaged DNA by a DNA glycosylase from Escherichia coli. Nucleic Acids Res 6: 3673 – 3684
Cohen NA, Egorin MJ, Snyder SW, Ashar B, Wietbarn BE,Pan SS, Ross DD, Hilton J (1991) Interaction of N,N’,N’-triethylenephosphoramide and N,N,N’-triethylenephosphoramide with cellular DNA. Cancer Res 51: 4360 – 4366
Dimple B, Harrison L (1994) Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 63: 915 – 948
Deutsch WA, Yacoub A, Jaruga P, Zastawny TH, Dizdaroglu M (1997) Characterization and mechanism of action of Drosophila ribosomal protein S3 DNA glycosylase activity for the removal of oxidatively damaged DNA bases. J Biol Chem 272: 32857 – 32860
Dinapoli RP, Brown LD, Arusell RM, Earle JD, O’Fallon JR, Buckner JC (1993) Phase III comparative evaluation of PCNU and carmustine combined with radiation therapy for high-grade glioma. J Clin Oncol 11: 1316 – 1321
Dixit M, Yang JL, Poirier MC, Price JO, Andrews PA, Arteaga CL (1997) Enhanced DNA repair as a mechanism of resistance to cisplatin in human ovarian cancer cells. J Biol Chem 272: 16455 – 16460
Dinh H, Popper HH, Buck CR, Zatloukal K (2003) Automated creation of a fully functional human chimeric DNA repair protein. J Biol Chem 278: 6789 – 6797
Graves RJ, Felzenszwalb I, Laval J, O’Connor TR (1992) Excision of 5-methylcytosine residues from DNA by a DNA N-methylcytosine DNA glycosylase from E. coli. Biochemistry 31: 12558 – 12562
Hansen WK, Deutsch WA, Yacoub A, Xu Y, Williams DA, Kelley MR (1998) Creation of a fully functional human chimeric DNA repair protein. Combining O6-methylguanine DNA methyltransferase (MGMT) and AP endonuclease (APE1) effector factor I, Ref1 DNA repair proteins. J Biol Chem 273: 756 – 762
Hansen WK, Kelley MR (2000) Review of mammalian DNA repair and translational implications. J Pharmacol Exp Ther 295: 1 – 9
Heimberger AB, Crotty LE, Archer GE, Hess KR, Wikstrand CJ, Friedman AH, Friedman HS, Bigner DD, Sampson JH (2003) Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. Clin Cancer Res 9(11): 4247 – 4254
Hoffmann M, Schmidt M, Wels W (1998) Activation of EGFR receptor family members suppresses the cytotoxic effects of tumor necrosis factor-alpha. Cancer Immunol Immunother 47: 167 – 175
Hui AB, Lo KW, Yin XL, Poon WS, Ng HK (2001) Detection of multiple gene mutations: multiplex PCR with normal base using array-based comparative genomic hybridization. Lab Invest 81: 717 – 723
Johnson SW, Perez RP, Godwin AK, Hamilton TC (1994) Role of platinum–DNA adduct formation and removal in cisplatin resistance in human ovarian cancer cell lines. Biochem Pharmacol 47: 689 – 697
Karaahil B, Girard PM, Boiteux S, Dizdaroglu M (1998) Substrate specificity of the Ogg1 protein of Saccharomyces cerevisiae: excision of guanine lesions produced in DNA by ionizing radiation- or hydrogen peroxide/metal ion-generated free radicals. Nucleic Acids Res 26: 1228 – 1233
Koi M, Umar A, Chauhan DP, Cherian SP, Carethers JM, Kunkel TA, Boland CR (1994) Human chromosome 3 correctly mismatch repair deficient and microsatellite instability and reduces N-methyl-N’-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. Cancer Res 54: 4308 – 4312
Kuga T, Sakamatsu S, Hiroyama Y, Kuroda H, Takahashi Y, Kusakabe T, Kato I, Nitsu Y (1997) Fibronectin fragment-facilitated retroviral transfer of the glutathione-S-transferase pi gene into CD34+ cells to protect them against alkylating agents. Hum Gene Ther 8: 1901 – 1910
Laemmli UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227: 680 – 685
Lai GM, Ozols RF, Smyth JF, Young RF, Hamilton TC (1988) Enhanced DNA repair and resistance to cisplatin in human ovarian cancer. Biochem Pharmacol 37: 4597 – 4600
Lei W, Mayotte JE, Levitt ML (1999) Enhancement of chemosensitivity and programmed cell death by tyrosine kinase inhibitors correlates with EGFR expression in non-small cell lung cancer cells. Anticancer Res 19: 221 – 228
Leighton C, Fisher B, Bauman G, Depiero S, Stitt L, Macdonald D, Cairns  G (1997) Supratentorial low-grade gliomas in adults: an analysis of prognostic factors and timing of radiation. J Clin Oncol 15: 1294 – 1301
Leung SY, Yuen ST, Chan TL, Chan AS, Ho JW, Kwan K, Fan YW, Hung KN, Chung LP, Wylie AH (2000) Chromosomal instability and p53 inactivation are required for genesis of glioblastoma but not for colorectal cancer in patients with germline mismatch repair gene mutation. Oncogene 20: 2156 – 2163
Liang K, Ang KK, Milas I, Hunter N, Fan Z (2003) The epidermal growth factor receptor mediates radioresistance. Int J Radiat Oncol Biol Phys 57(1): 246 – 254
Limp-Foster M, Kelley MR (2000) DNA repair and gene therapy: implications for translational use. Environ Mol Mutagen 35: 71 – 81
Litz-Jackson S, Miller AH, Burgess GS, Boswell HS (1992) Dissection of nuclear events on p21 RAS transformation of FDC-P1 myeloid cells; c-jun/PAI expression versus c-myc transcription. Blood 79: 2404 – 2414
Lydon NB, Mett H, Mueller M, Cozier N, Stover D, Daniels D, Traxler P, Buchdunger E (1998) A potent protein-tyrosine kinase inhibitor which selectively blocks proliferation of epidermal growth factor receptor-expressing tumor cells in vitro and in vivo. Int J Cancer 76: 154 – 163
Mello JA, Acharya S, Fishe1 R, Essigmann JM (1996) The mismatch-repair protein MSH2 binds selectively to DNA adducts of the antitumor drug cisplatin. Chem Biol 3: 579 – 589
Mitra S, Hazra TK, Roy B, Ibeda S, Biswas T, Lock J, Boldogh I, Izumi T (1997) Complexities of DNA base excision repair in mammalian cells. Mol Cell 7: 305 – 312
Modjtabahi H, Affleck K, Forbes D, Dean C (1998) EGFR blockade by tyrosine kinase inhibitor or monoclonal antibody inhibits growth, directs terminal differentiation and induces apoptosis in the human squamous cell carcinoma HNS. Int J Oncol 13: 335 – 342
Möhrenweiser HW, Wilson III DM, Jones IM (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res* 526: 93–125

O’Neill JP (2000) DNA damage, DNA repair, cell proliferation, and DNA replication: how do gene mutations result? *Proc Natl Acad Sci USA* 97: 11137–11139

Park S-J, Oh E-J, Yoo M-A, Lee S-H (2001) Involvement of DNA-dependent protein kinase in regulation of stress-induced JNK activation. *DNA Cell Biol* 20: 637–645

Perez M, Donato NJ (1996) Activation of epidermal growth factor receptor tyrosine phosphorylation by tumor necrosis factor correlates with loss of cytotoxic activity. *J Interferon Cytokine Res* 16: 307–314

Robertson KA, Bullock HA, Xu Y, Tritt R, Zimmerman E, Ulbright TM, Foster RS, Einhorn LH, Kelley MR (2001) Altered expression of Ape1/ref-1 in germ cell tumors and overexpression in NT2 cells confers resistance to bleomycin and radiation. *Cancer Res* 61: 2220–2225

Rosenquist TA, Zharkov DO, Grollman AP (1997) Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc Natl Acad Sci USA* 94: 7429–7434

Skadel M, Ormsby AH, Pettay JD, Tsiftakis EK, Liou LS, Klein EA, Levin HS, Zippe CD, Tubbs RR (2001) Aneusomy of chromosomes 7, 8, and 17 and amplification of HER-2/neu and epidermal growth factor receptor in Gleason score 7 prostate carcinoma: a differential fluorescent *in situ* hybridization study of Gleason pattern 3 and 4 using tissue microarray. *Hum Pathol* 32: 1392–1397

Stigter E, Drissi R., Lee S-H (1998) Functional analysis of human replication protein A in nucleotide excision repair. *J Biol Chem* 273: 9337–9343

Umar A, Koi M, Risinger JI, Glaab WE, Tindall KR, Kolodner RD, Boland CR, Barrett JC, Kunkel TA (1997) Correction of hypermutability, N-methyl-N-nitro-N-nitrosoguanidine resistance, and defective DNA mismatch repair by introducing chromosome 2 into human tumor cells with mutations in MSH2 and MSH6. *Cancer Res* 57: 3949–3955

van Puijenbroek AA, van Weering DH, van den Brink CE, Bos JL, van der Saag PT, de Laat SW, den Hertog J (1997) Cell scattering of SK-N-MC neuroepithelioma cells in response to Ret and FGF receptor tyrosine kinase activation is correlated with sustained ERK2 activation. *Oncogene* 14: 1147–1157

Wang X, Huong SM, Chiu ML, Raab-Traub N, Huang ES (2003) Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. *Nature* 424(6947): 456–461

Waters TR, Gallinari P, Jiriczky J, Swann PF (1999) Human thymine DNA glycosylase binds to apurinic sites in DNA but is displaced by human apurinic endonuclease 1. *J Biol Chem* 274: 67–74

Witters L, Kumar R, Mandal M, Bennett CF, Miraglia L, Lipton A (1999) Antisense oligonucleotides to the epidermal growth factor receptor. *Breast Cancer Res Treat* 53: 41–50

Zhang HT, Wang Q, Greene MI, Murali R (2000) New perspectives on anti-HER2/neu therapeutics. *Drug News Perspect* 13(6): 325–329

Zharkov DO, Rosenquist TA, Gerchman SE, Grollman AP (2000) Substrate specificity and reaction mechanism of murine 8-oxoguanine-DNA glycosylase. *J Biol Chem* 275: 28607–28617