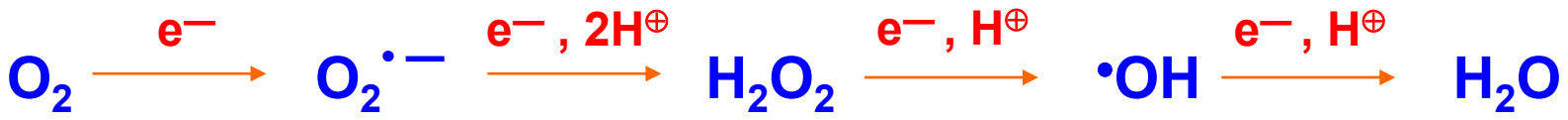
National Institute of Standards and Technology
Gaithersburg, Maryland, USA

Oxidatively Induced DNA Damage
Mechanisms, Cellular Repair and Disease

Miral Dizdaroglu

Formation of oxygen-derived species

The unpaired orbitals of dioxygen (•O=O•) can sequentially accommodate single electrons to yield superoxide radical, hydrogen peroxide, hydroxyl radical and water.



The major intracellular source of these species is leakage from the mitochondrial electron-transport chain. Other intracellular processes can also result in the generation of oxygen-derived species.

Reviewed in:
Halliwell, B. and Gutteridge, J. M. C., *Free Radicals in Biology and Medicine*, 2007

Transition metal ion-catalyzed Haber-Weiss reaction

$$O_2^{\bullet -} + Fe(III) \longrightarrow O_2 + Fe(II)$$

$$H_2O_2 + Fe(II) \longrightarrow {}^{\bullet}OH + OH^- + Fe(III)$$

$$O_2^{\bullet -} + H_2O_2 \longrightarrow \boxed{{}^{\bullet}OH} + OH^- + O_2$$

Interaction of ionizing radiation with water in living cells

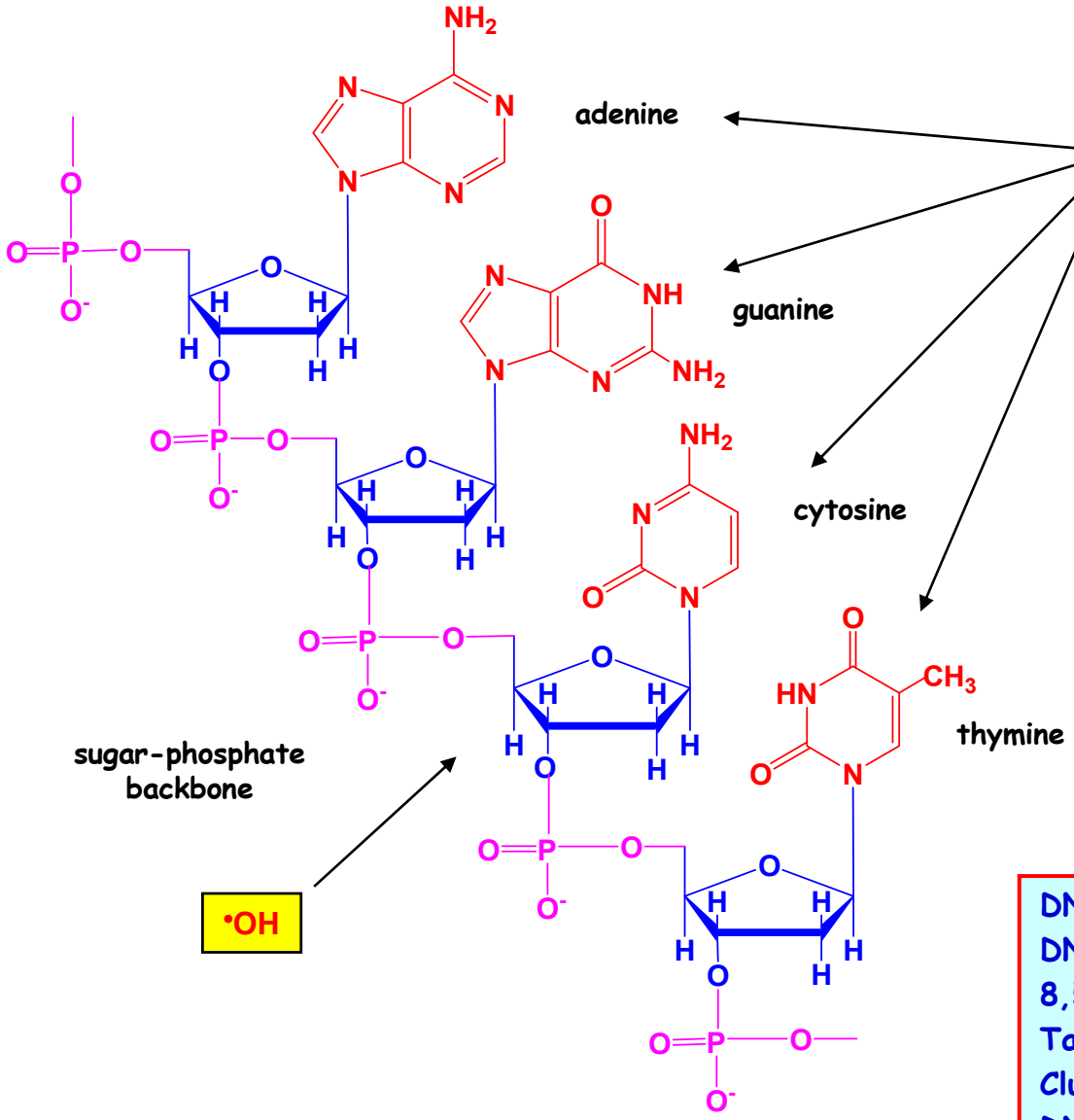
$$H_2O \xrightarrow{\textit{ionizing radiation}} \boxed{{}^{\bullet}OH},\ e_{aq}^-,\ H^{\bullet},\ O_2^{\bullet -},\ H_2O_2,\ H_2$$

Hydroxyl radical reacts with DNA constituents near or at diffusion-controlled reaction rates

($k \approx 5–10 \times 10^9\ M^{-1}\ s^{-1}$)

Reviewed in:
Halliwell, B. and Gutteridge, J. M. C., *Free Radicals in Biology and Medicine*, 2007

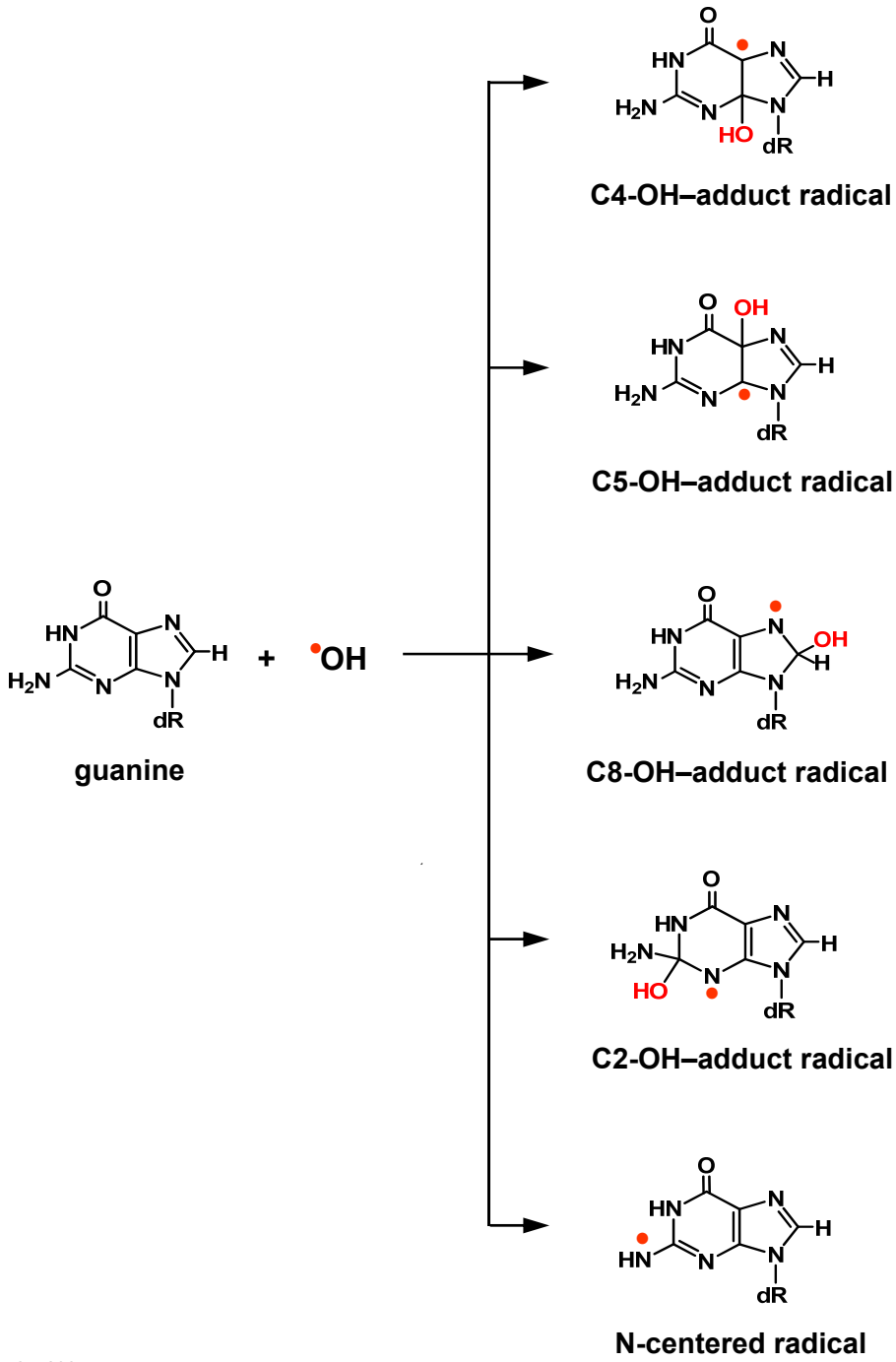
Sites of oxidatively induced damage in DNA



DNA base damage
DNA sugar damage
8,5'-cyclopurine-2'-deoxynucleosides
Tandem lesions
Clustered sites
DNA-protein cross-links
Single- and double-strand breaks
Abasic sites

Reviewed in:
Evans, M. D., Dizdaroglu, M. and Cooke, M. S., *Mutat. Res.* 567, 1-61, 2004
Dizdaroglu, M. and Jaruga, *Free Radic. Res.* 46, 382-419, 2012

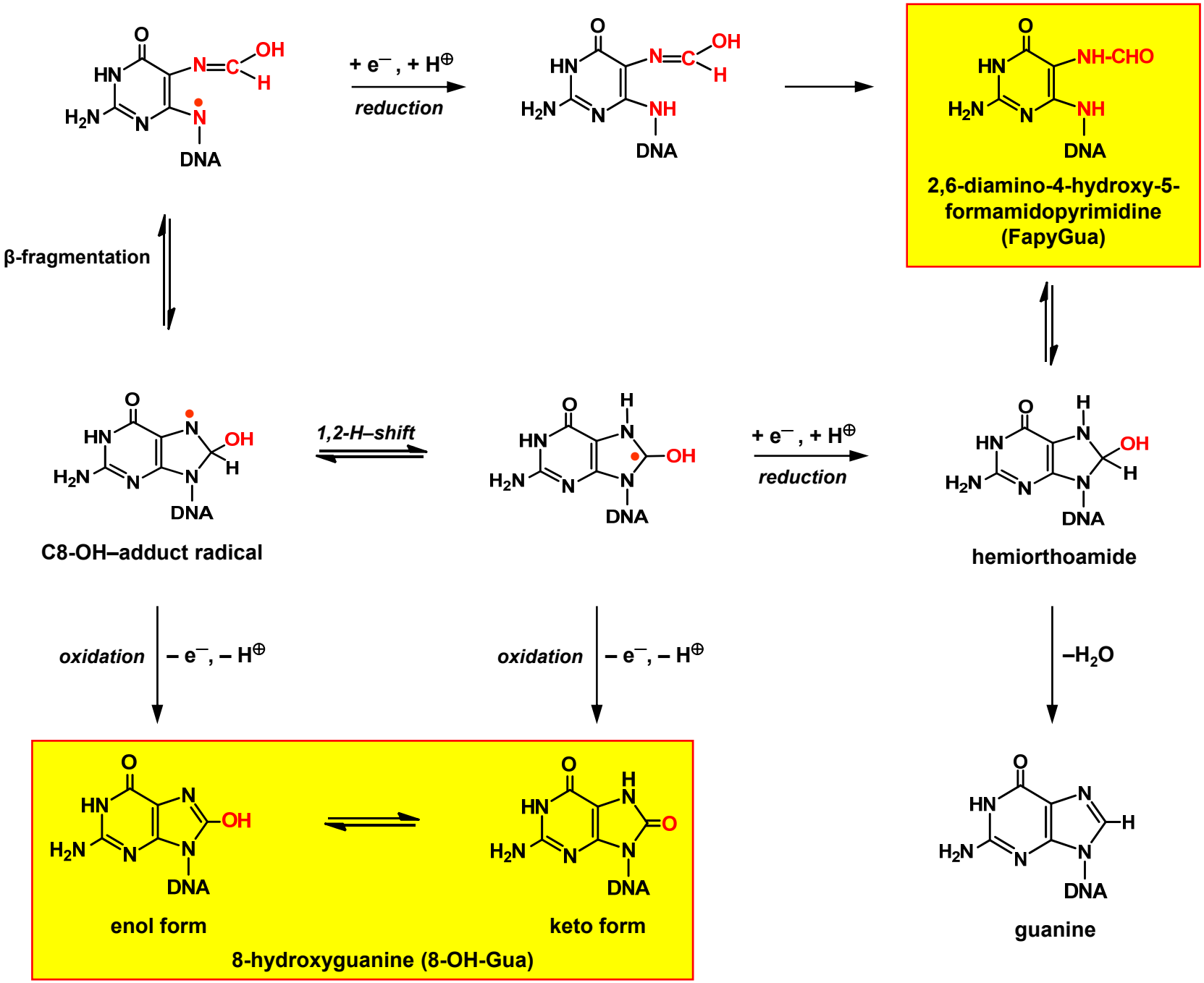
Reactions of hydroxyl radical with purines



Reviewed in:

Evans, M. D., Dizdaroglu, M. and Cooke, M. S., *Mutat. Res.* 567, 1-61, 2004
Dizdaroglu, M. and Jaruga, *Free Radic. Res.* 46, 382-419, 2012

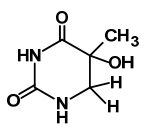
Formation of guanine products

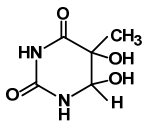


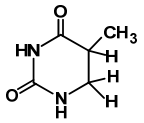
Adenine undergoes analogous reactions leading to analogous products

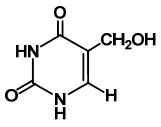
Reviewed in:
Dizdaroglu, M. and Jaruga, P., *Free Radic. Res.* 46, 382-419, 2012

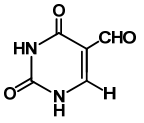
Products of oxidatively induced damage to DNA bases

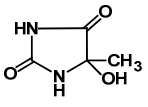

5-hydroxy-6-hydro-thymine

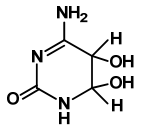

thymine glycol

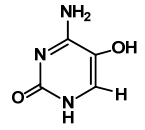

5,6-dihydrothymine

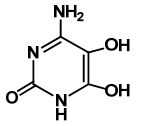

5-hydroxymethyl-uracil

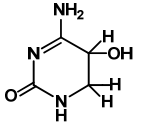

5-formyluracil

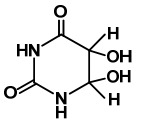

5-hydroxy-5-methyl-hydantoin

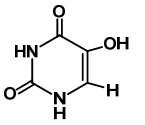

cytosine glycol

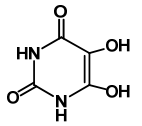

5-hydroxycytosine

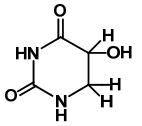

5,6-dihydroxycytosine

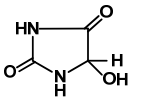

5-hydroxy-6-hydro-cytosine

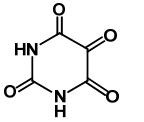

uracil glycol

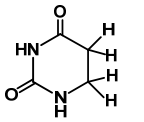

5-hydroxyuracil

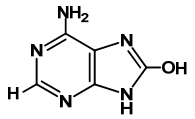

5,6-dihydroxyuracil

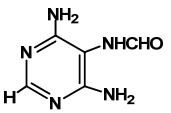

5-hydroxy-6-hydrouracil

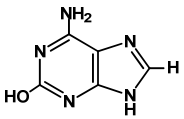

5-hydroxyhydantoin

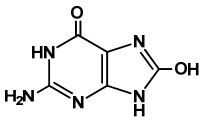

alloxan

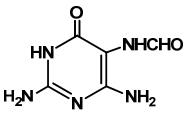

5,6-dihydrouracil

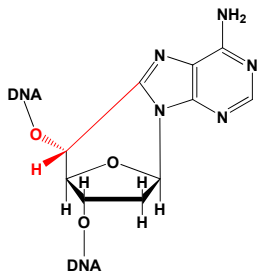

8-hydroxyadenine

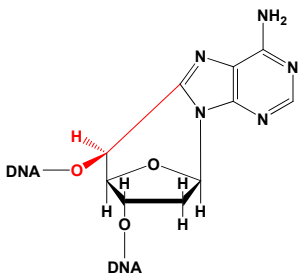

4,6-diamino-5-form-amidopyrimidine

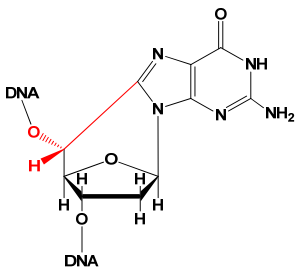

2-hydroxyadenine

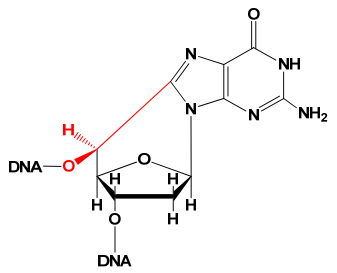

8-hydroxyguanine


2,6-diamino-4-hydroxy-5-formamidopyrimidine


(5'*R*)-8,5'-cyclo-2'-deoxyadenosine


(5'*S*)-8,5'-cyclo-2'-deoxyadenosine


(5'*R*)-8,5'-cyclo-2'-deoxyguanosine


(5'*S*)-8,5'-cyclo-2'-deoxyguanosine

Reviewed in:
Evans, M.D., Dizdaroglu, M. and Cooke, M.S., *Mutat. Res.* 567, 1-61, 2004
Dizdaroglu, M. and Jaruga, P., *Free Radic. Res.* 46, 382-419, 2012

Techniques used for the measurement of DNA damage and repair

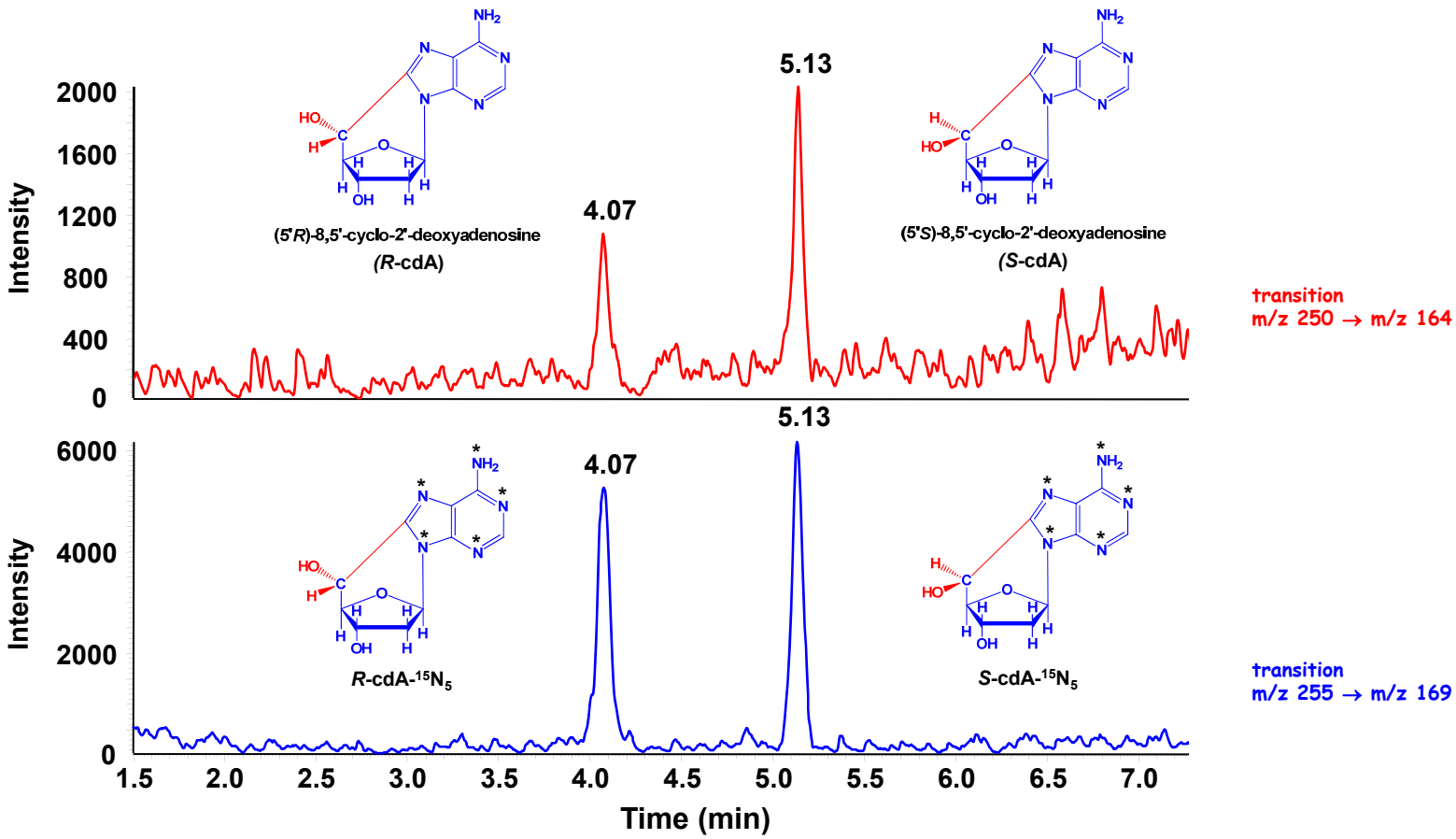
Gas chromatography/mass spectrometry (GC/MS) with isotope-dilution

Liquid chromatography/mass spectrometry (LC/MS) with isotope-dilution

Gas chromatography/tandem mass spectrometry (GC-MS/MS) with isotope-dilution

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) with isotope-dilution

Measurement of (5'*R*)-and (5'*S*)-8,5'-cyclo-2'-deoxyadenosines *in vivo* by LC-MS/MS



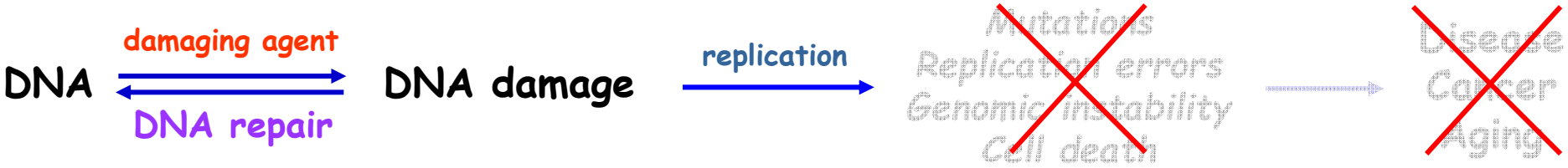
Levels in mouse liver (*n* = 10)

***R*-cdA**, 1.33±0.24 molecules/10^8 DNA nucleotides

***S*-cdA**, 4.82±0.65 molecules/10^8 DNA nucleotides

Jaruga, P., Xiao, Y., Nelson, B. C. and Dizdaroglu, M., *Biochem. Biophys. Res. Commun.* 386, 656-660, 2009

DNA damage, repair and consequences

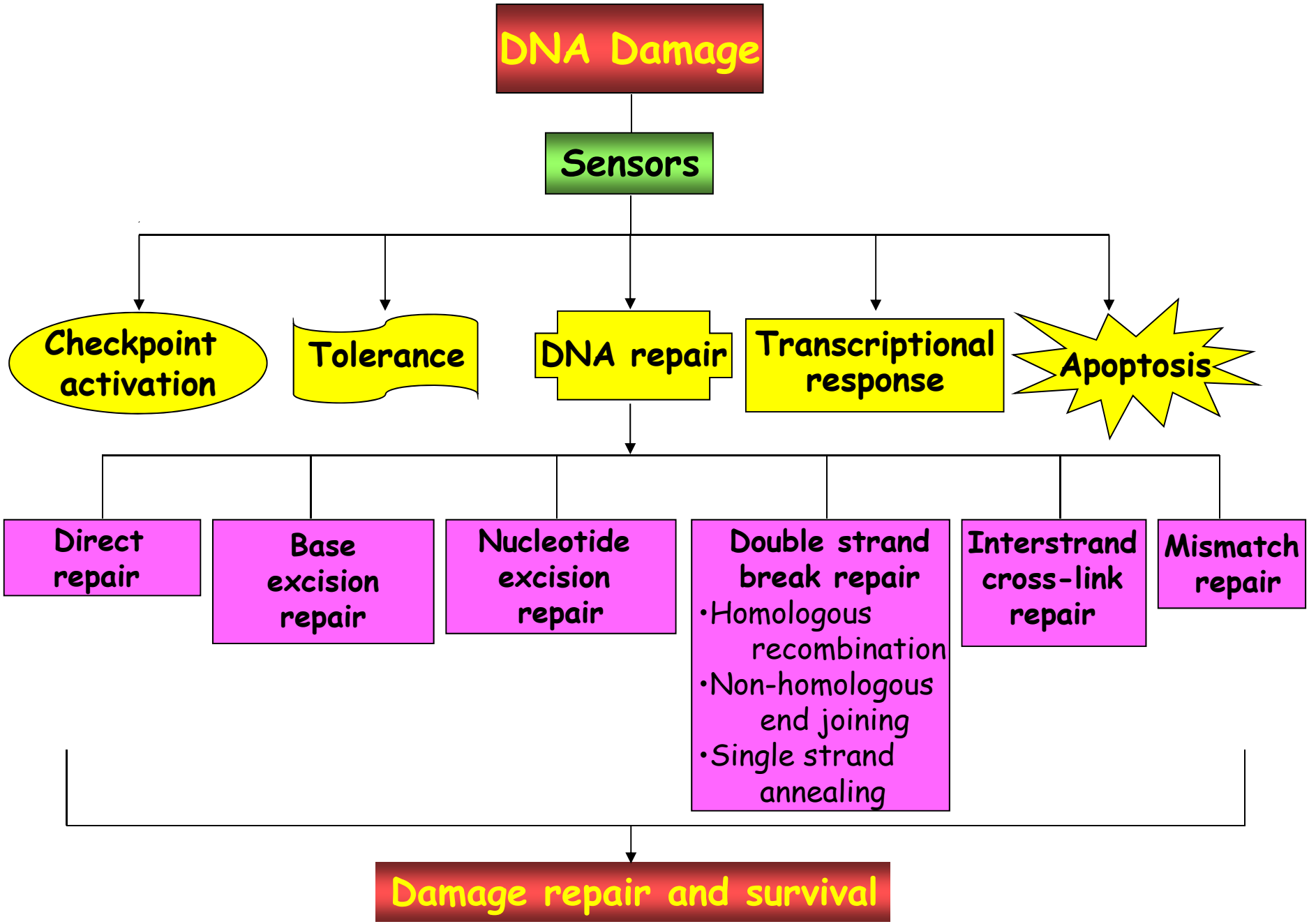


DNA damage ultimately causes 80 to 90 percent of human cancers

R. Doll and R. Peto, *J. Natl. Cancer Inst.* 66, 1192 (1981)

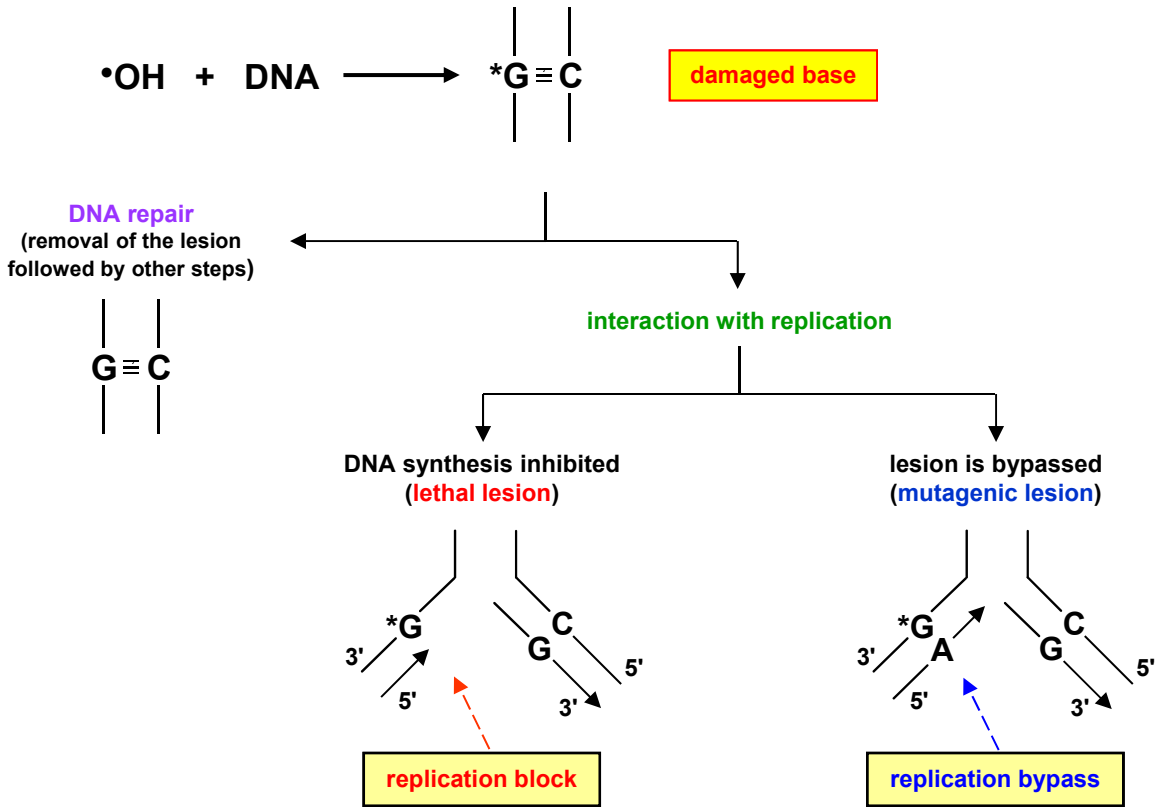
There is an increasing awareness of the relevance of DNA damage and repair to human health

Biological responses to genomic DNA damage



Reviewed in:
Madhusudan, S. and Middleton, M. R., *Cancer Treatment Reviews* 31, 603–617, 2005

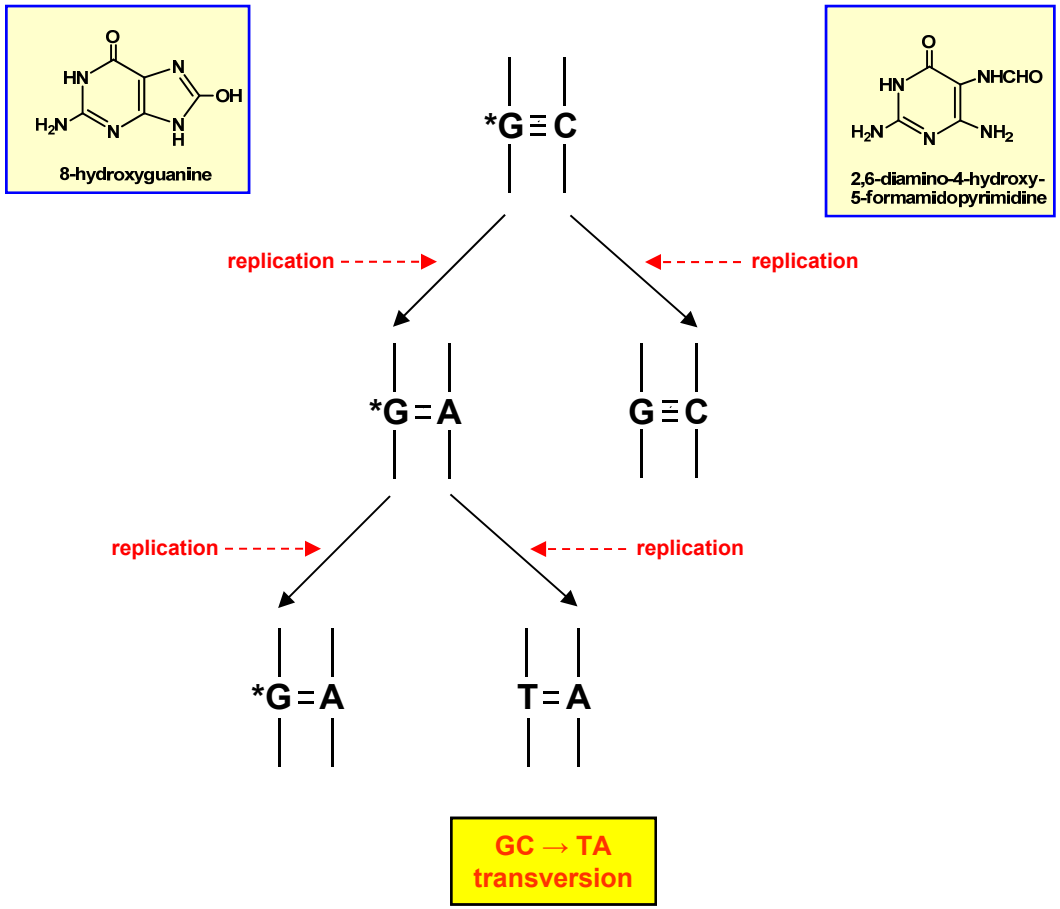
Pathways for processing of a DNA lesion in living cells



It is the interplay between these three events that determines the ultimate future the damaged cell

Reviewed in:
Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A. and Ellenberger, T., *DNA Repair and Mutagenesis*, 2006

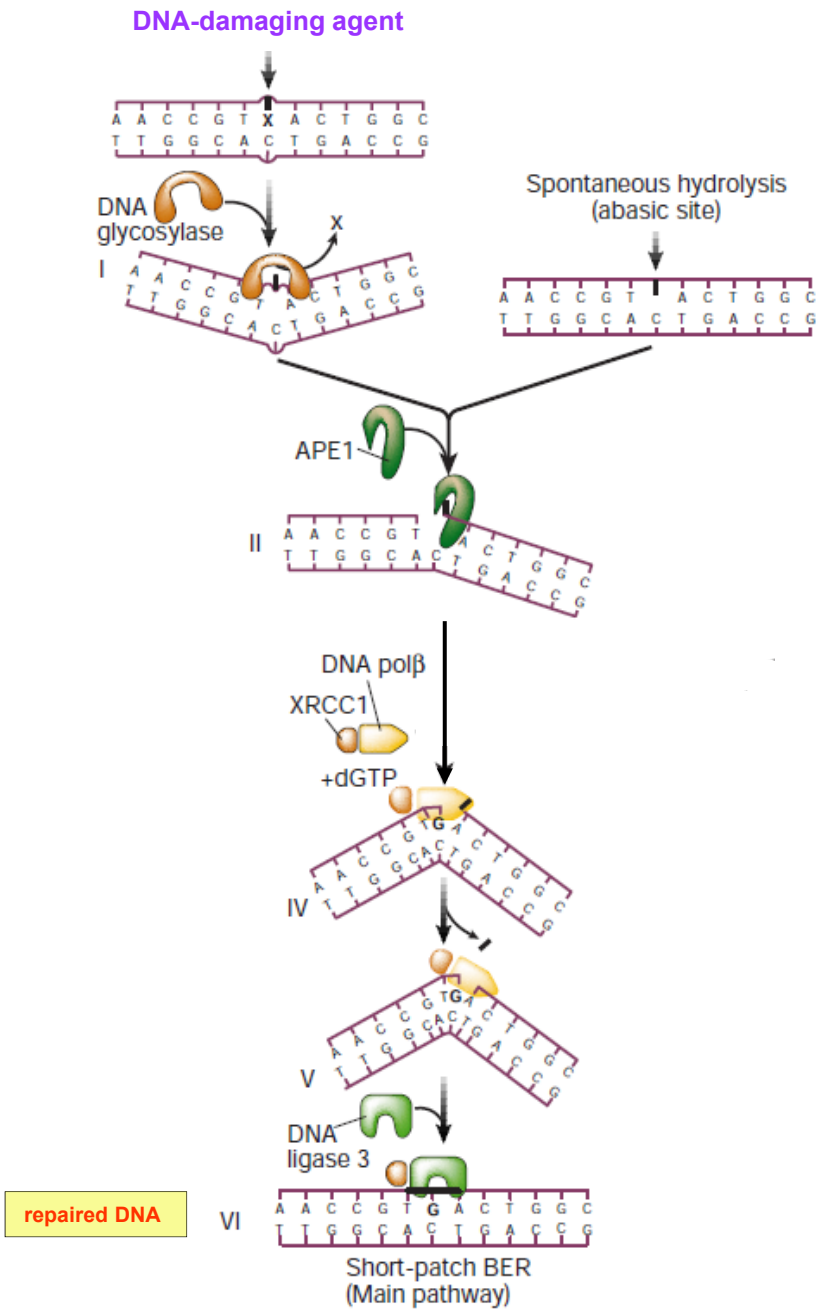
Mutagenesis by products of DNA damage

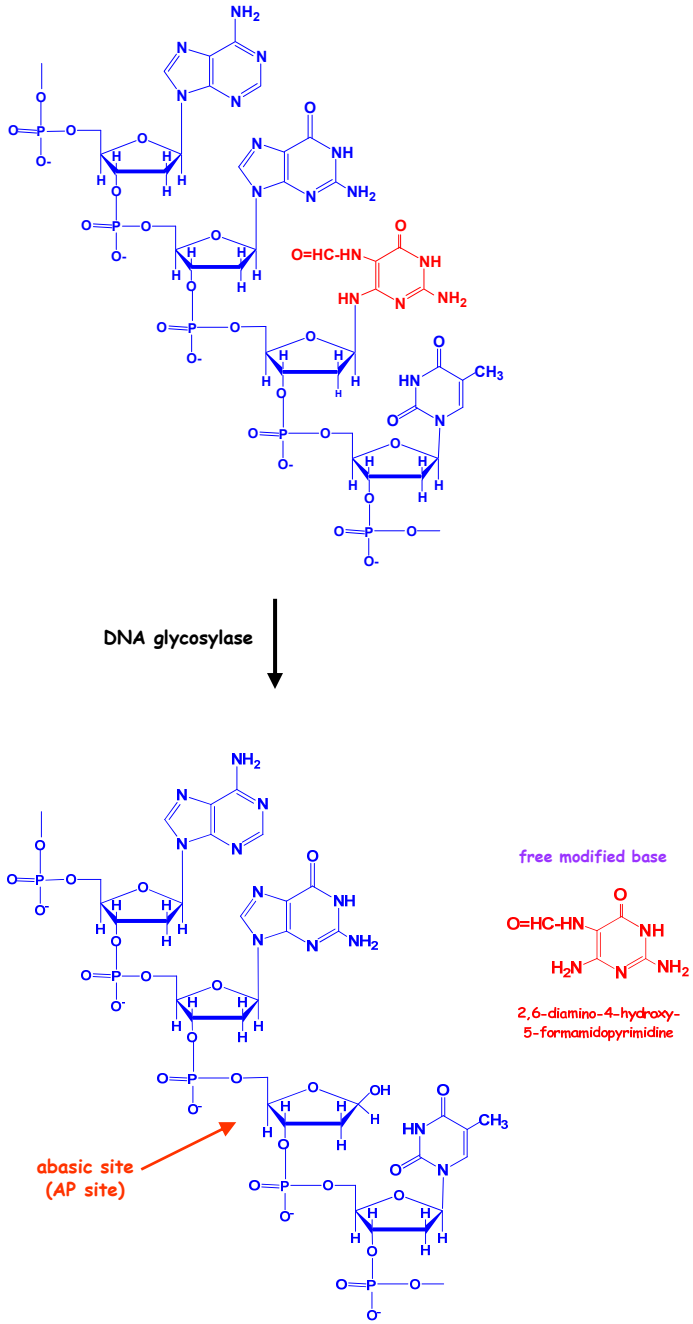


GC→TA transversions are the second most common somatic mutations found in human cancers with 14.6% of all mutations in the tumor suppressor gene *TP53*

Wood, M.L., Dizdaroglu, M., Gajewski, E. and Essigmann, J.M., *Biochemistry* 29, 7024-7032, 1990
Wiederholt, C.J. and Greenberg, M.M., *J. Am. Chem. Soc.* 124, 7278-7679, 2002

Mechanisms of base excision repair





Reviewed in:
Hoeijmakers, J.H.J., *Nature* 411, 366-374, 2001
Friedberg, E.C., Walker, G.C., Siede, W., Wood, R.D., Schultz, R.A. and Ellenberger, T., *DNA Repair and Mutagenesis*, 2006

DNA glycosylases in base excision repair of DNA damage

E. coli enzymes

Formamidopyrimidine glycosylase (Fpg or MutM)
Endonuclease III (Nth)
Endonuclease VIII (Nei)

Eukaryotic enzymes

OGG1
α-hOGG1-Ser^{326} and α-hOGG1-Cys^{326}
β-hOGG1
S. cerevisiae OGG1
Drosophila melanogaster OGG1
Arabidopsis thaliana OGG1

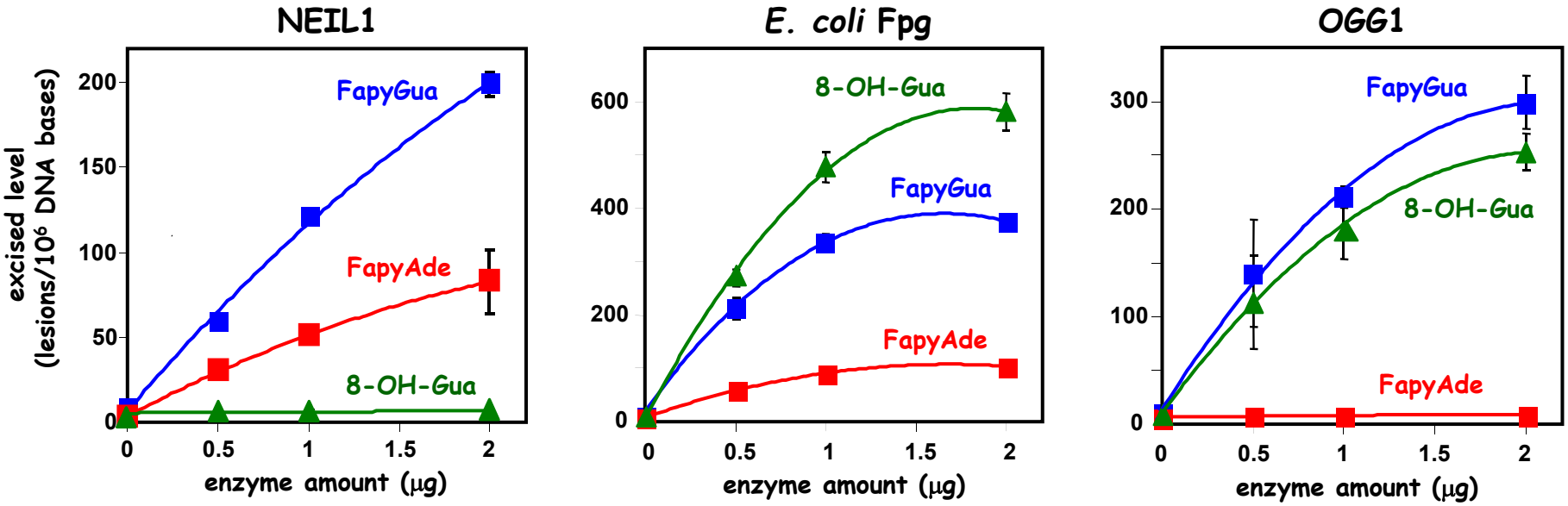
NTH1 (human), Nth (others)
hNTH1
S. Pombe Nth

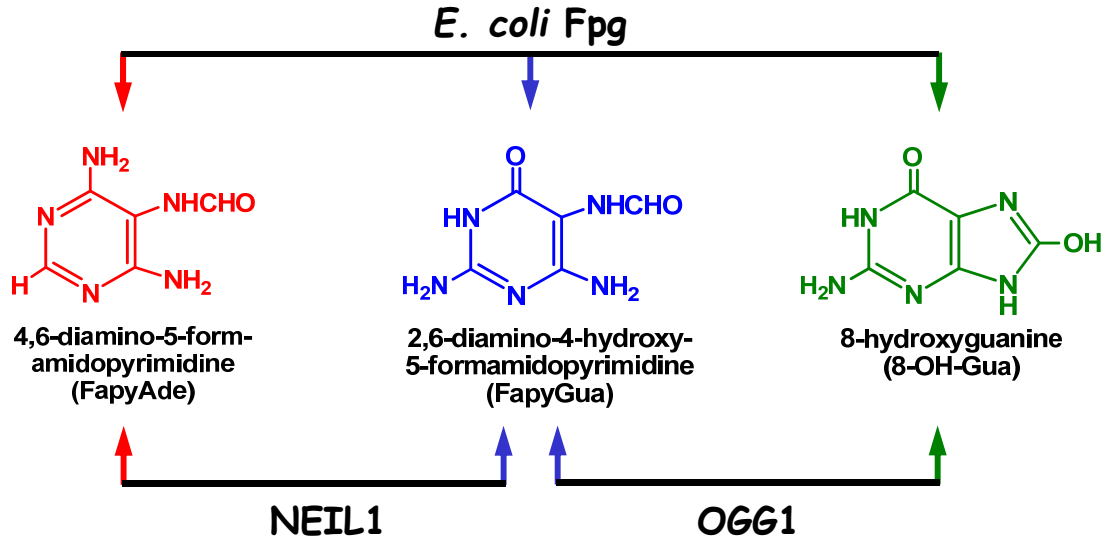
Ntg1 and Ntg2 (*S. cerevisiae*)

NEIL1, NEIL2, NEIL3

Reviewed in:
Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A. and Ellenberger, T., *DNA Repair and Mutagenesis*, 2006

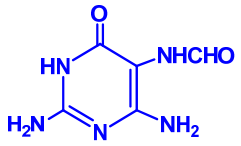
Different substrate specificities of NEIL1, Fpg and OGG1



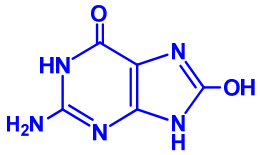


Reviewed in:
Dizdaroglu, M., *Mutat. Res.* 591, 45-59, 2005

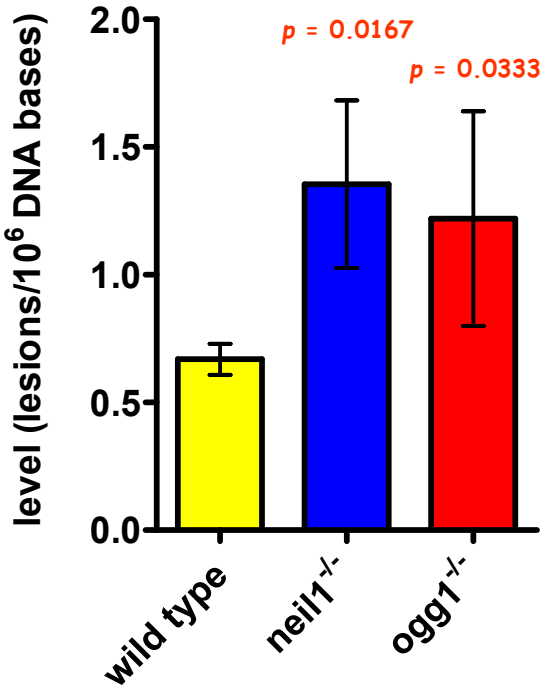
Accumulation of FapyGua and 8-OH-Gua in liver of *neil1*$^{-/-}$ and *ogg1*$^{-/-}$ mice

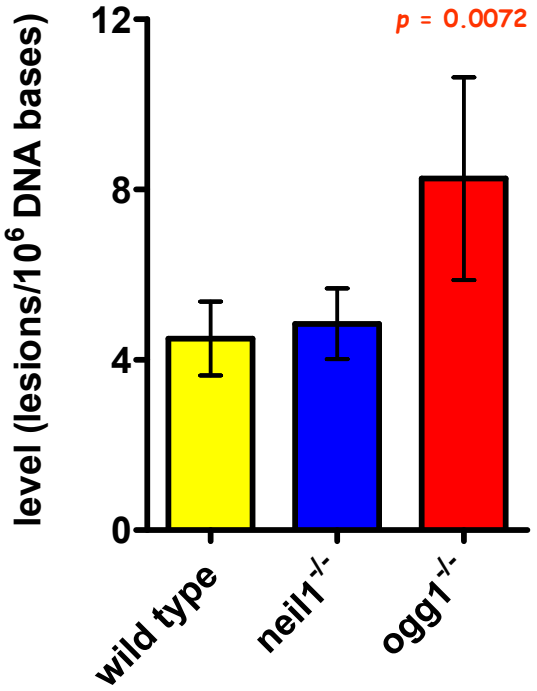


2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua)



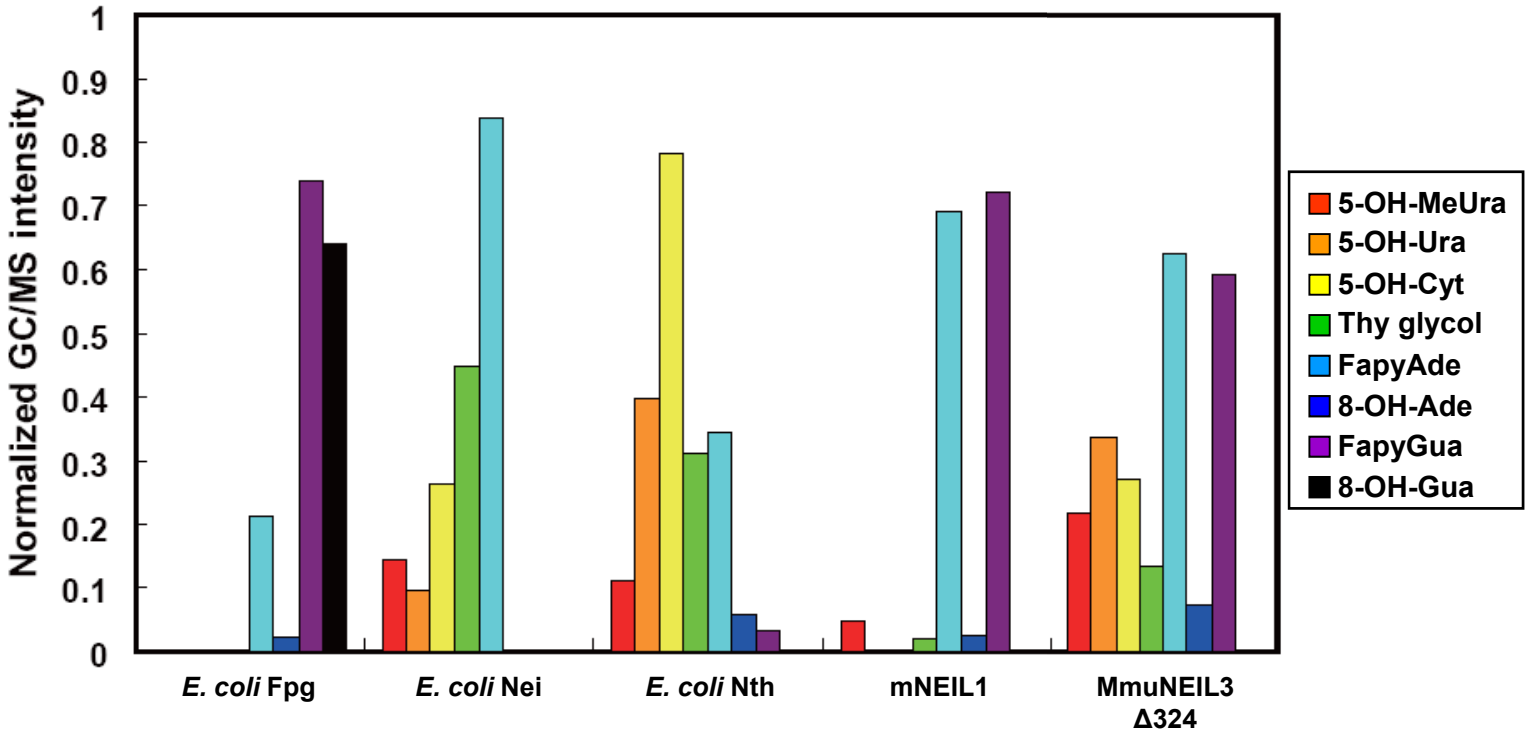
8-hydroxyguanine (8-OH-Gua)





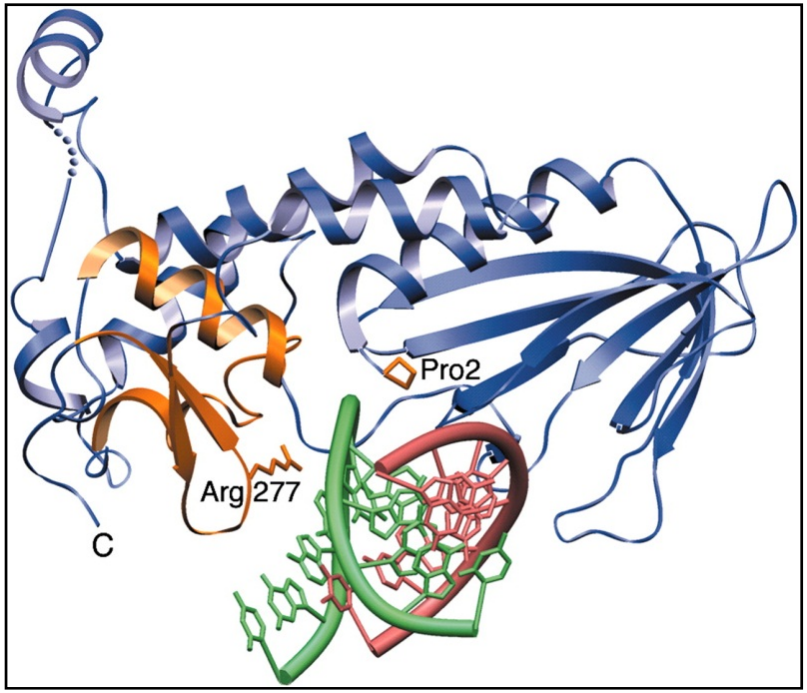
Jaruga, P., Xiao, Y., Vartanian, V., Lloyd, R. S. and Dizdaroglu, M., *Biochemistry* 49, 1053-1055, 2010

Substrates of DNA glycosylases



Liu, M., Bandaru, V., Bond, J. P., Jaruga, P., Zhao, X., Christov, P. P., Burrows, C. J., Rizzo, C. J., Dizdaroglu, M. and Wallace, S. S., *Proc. Natl. Acad. Sci. USA* 107, 4925-4930, 2010

Human NEIL1 protein



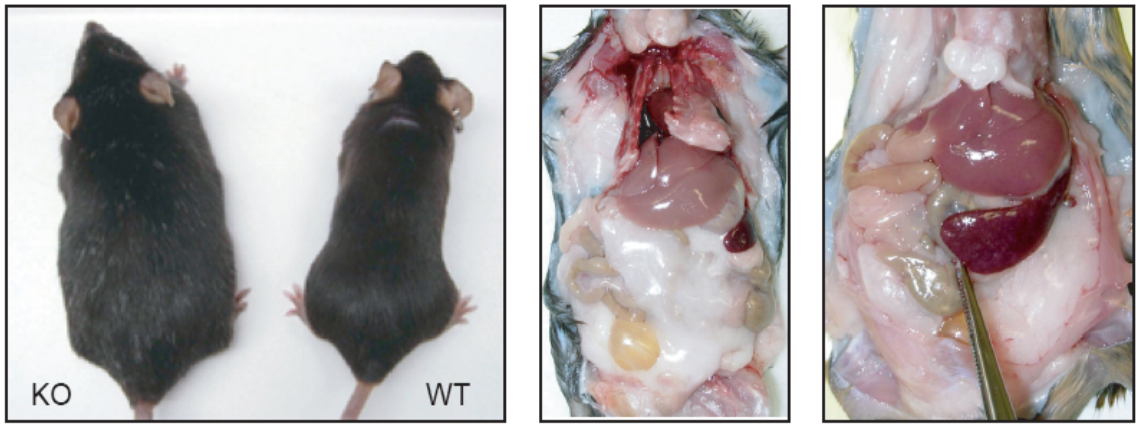

```
        10         20         30         40         50         60
MPEGPELHLA SQFVNEACRA LVFGGCVEKS SVSRNPEVPF ESSAYRISAS ARGKELRLIL
        70         80         90        100        110        120
SPLPGAQPQQ EPLALVFRFG MSGSFQLVPR EELPRHAHLR FYTAPPGPRL ALCFVDIRRF
       130        140        150        160        170        180
GRWDLGGKWQ PGRGPCVLQE YQQFRENVLR NLADKAFDRP ICEALLDQRF FNGIGNYLRA
       190        200        210        220        230        240
EILYRLKIPP FEKARSVLEA LQQHRPSPEL TLSQKIRTKL QNPDLLELCH SVPKEVVQLG
       250        260        270        280        290        300
GRGYGSESGE EDFAAFRAWL RCYGMPGMSS LQDRHGRTIW FQGDPGPLAP KGRKSRKKKS
       310        320        330        340        350        360
KATQLSPEDR VEDALPPSKA PSRTRRAKRD LPKRTATQRP EGTSLQQDPE APTVPKKGRR
       370        380        390
KGRQAASGHC RPRKVKADIP SLEPEGTSAS
```

Molecular mass 43.6 kDa

Hazra, T.K., Izumi, T., Boldogh, I., Imhoff, B., Kow, Y.W., Pawel Jaruga, P., Dizdaroglu, M. and Mitra, S., *Proc. Natl. Acad. Sci. USA* 99, 3523-3528, 2002
Doublie, S., Bandaru, V., Bond, J.P. and Wallace, S.S., *Proc. Natl. Acad. Sci. USA* 101, 10284-10289, 2004

Role of NEIL1 in disease

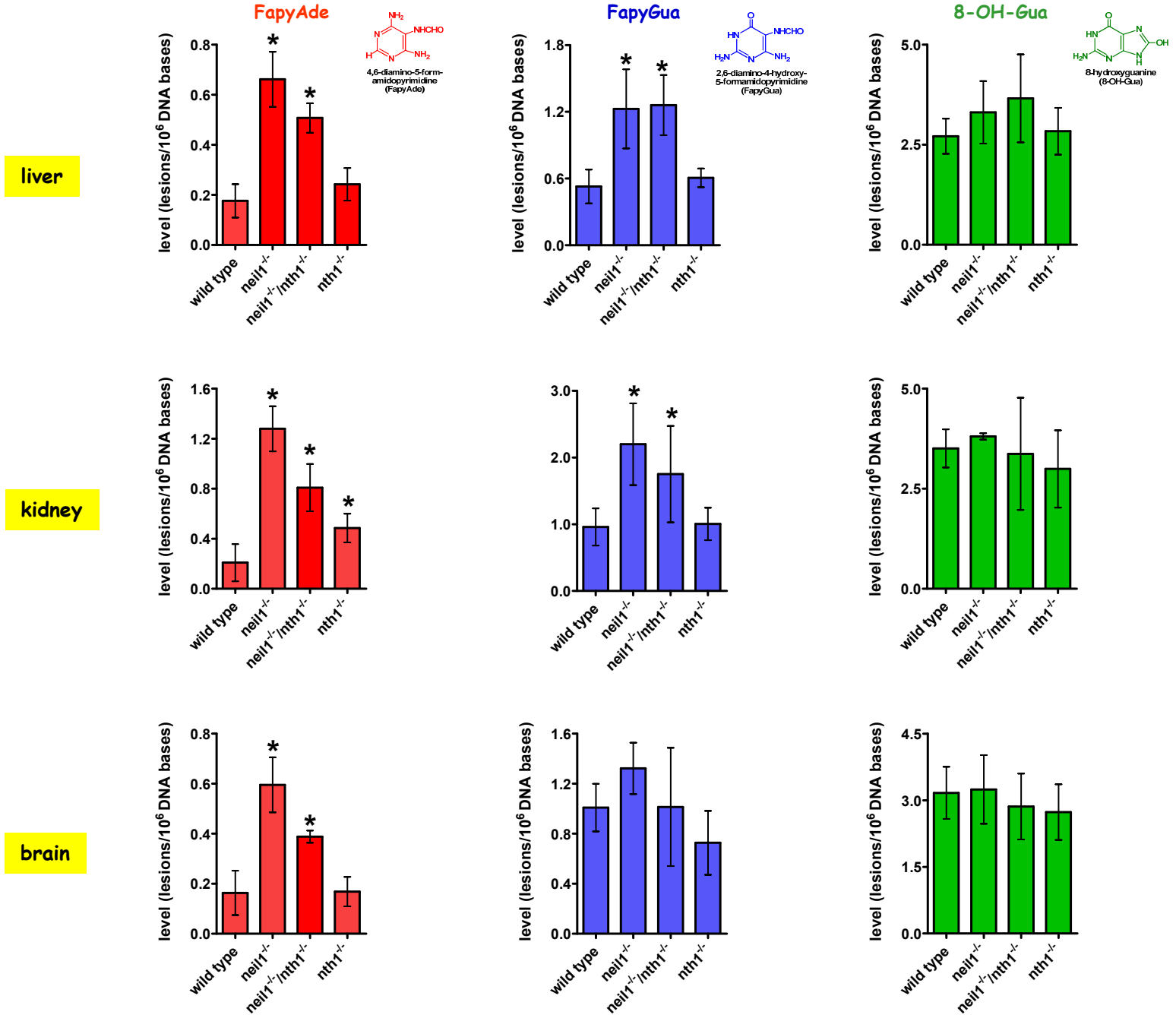
- Several mutated and polymorphic forms of NEIL1 have been discovered in humans.
- Studies showed a correlation of inactivating mutations in *neil1* gene with human gastric cancer
- Cells deficient in NEIL1 are sensitized to killing effects of ionizing radiation.
- Expression of NEIL1 is elevated after oxidative stress.
- NEIL1 exists in both the nucleus and mitochondrion, suggesting an overall involvement in the maintenance of the genomic stability.
- Without oxidative stress, many *neil1*$^{-/-}$ mice (predominantly males) develop severe obesity, dyslipidemia, fatty liver disease and hyperinsulinemia. In humans, this combination of clinical manifestations including hypertension is known as the *metabolic syndrome*, and is estimated to affect more than 40 million people in the USA alone.



- *Neil1*$^{-/-}$ mice exhibit late onset of certain cancers

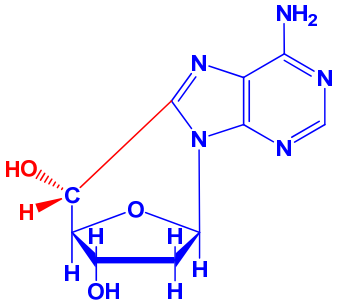
Vartanian, V., Lowell, B., Minko1, I.G., Wood, T.G., Ceci, J.C., George, S., Ballinger, S.W., Corless, C.L., McCullough, A.K, and Lloyd, R.S., *Proc. Natl. Acad. Sci. USA* 108, 1864-1869, 2006

Accumulation of DNA lesions in *neil1*$^{-/-}$, *nth1*$^{-/-}$ and *neil1*$^{-/-}$/*nth1*$^{-/-}$ mice

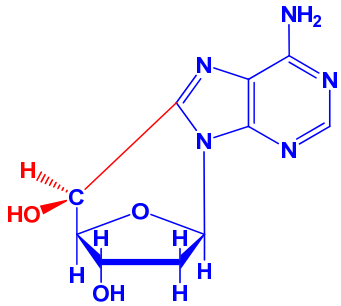


Chan, M. K., Ocampo-Hafalla. M. T., Vartanian, V., Jaruga, P., Kirkali, G., Koenig, K. L., Brown, S., Lloyd, R. S., Dizdaroglu, M. and Teebor, G.W., *DNA Repair* 8, 786-794, 2009

(5'*R*)- and (5'*S*)-8,5'-cyclo-2'-deoxyadenosines accumulate in liver of *neil1*$^{-/-}$ mice

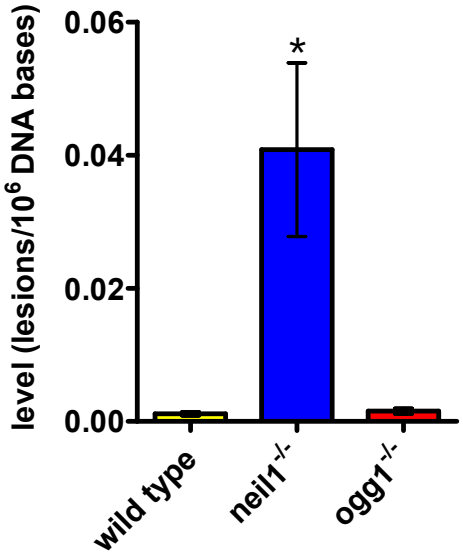


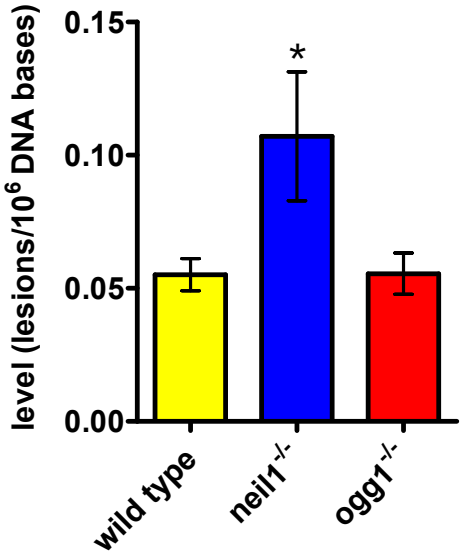
(5'*R*)-8,5'-cyclo-2'-deoxyadenosine



(5'*S*)-8,5'-cyclo-2'-deoxyadenosine

Repaired by nucleotide excision repair

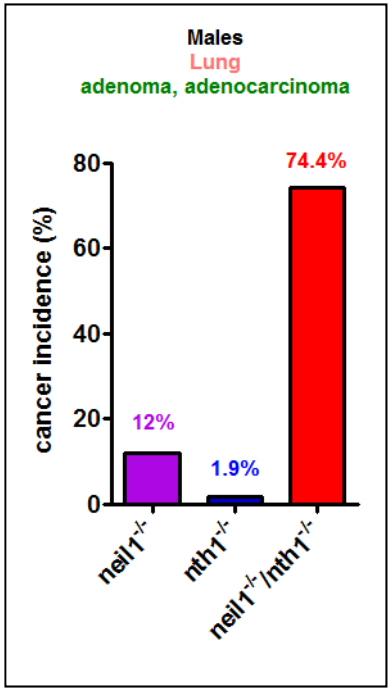




Does NEIL1 play a role in nucleotide excision repair?

Jaruga, P., Xiao, Y., Vartanian, V., Lloyd, R. S. and Dizdaroglu, M., *Biochemistry* 49, 1053-1055, 2010

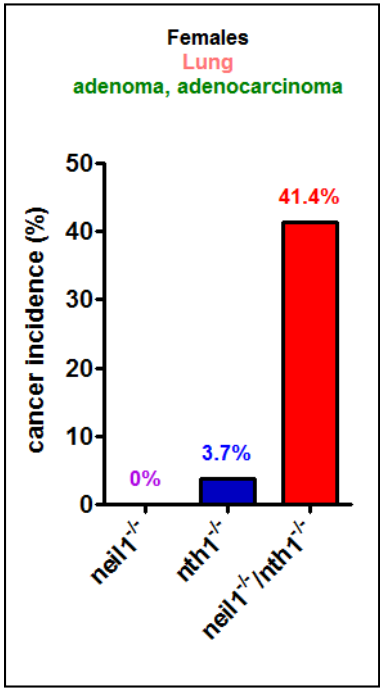
Cancer incidence in *nth1*$^{-/-}$, *neil1*$^{-/-}$ and *nth1*$^{-/-}$/*neil1*$^{-/-}$ mice



neil1$^{-/-}$ (n = 25)

nth1$^{-/-}$ (n = 52

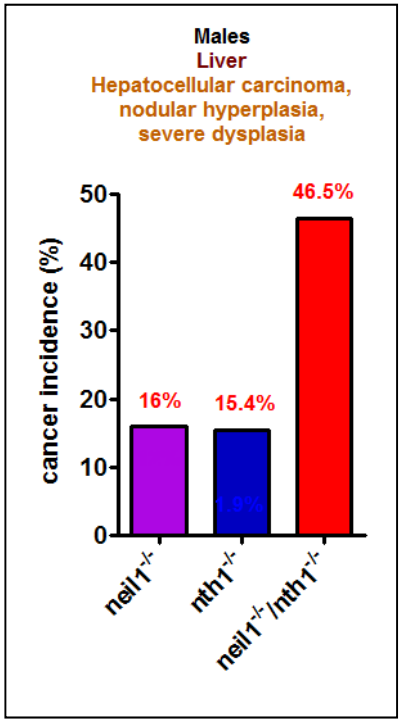
neil1$^{-/-}$/*nth1*$^{-/-}$ (n = 43)



neil1$^{-/-}$ (n = 18)

nth1$^{-/-}$ (n = 54)

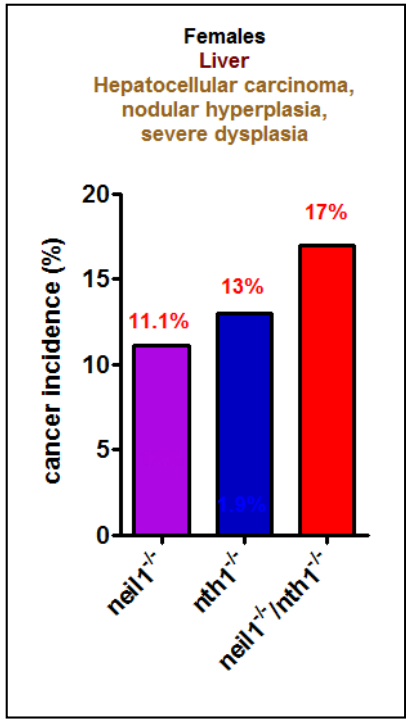
neil1$^{-/-}$/*nth1*$^{-/-}$ (n = 29)



neil1$^{-/-}$ (n = 25)

nth1$^{-/-}$ (n = 52)

neil1$^{-/-}$/*nth1*$^{-/-}$ (n = 43)



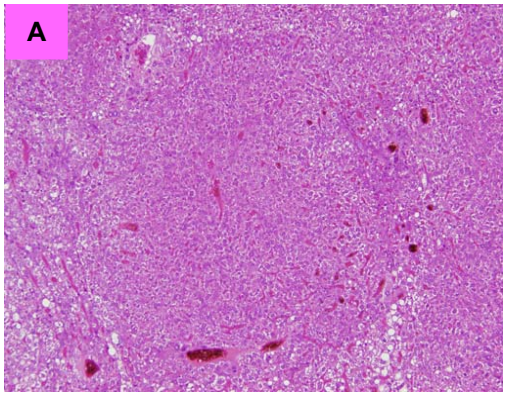
neil1$^{-/-}$ (n = 18)

nth1$^{-/-}$ (n = 54)

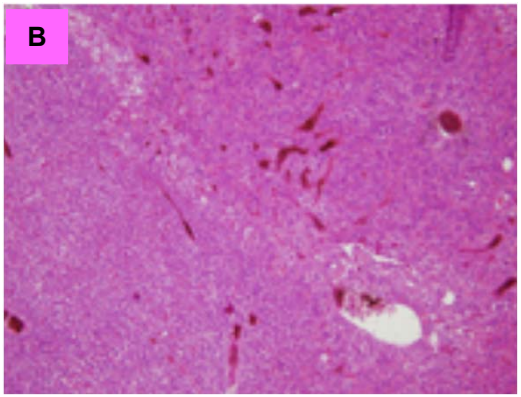
neil1$^{-/-}$/*nth1*$^{-/-}$ (n = 29)

Chan, M. K., Ocampo-Hafalla. M. T., Vartanian, V., Jaruga, P., Kirkali, G., Koenig, K. L., Brown, S., Lloyd, R. S., Dizdaroglu, M. and Teebor, G.W., *DNA Repair* 8, 786-794, 2009

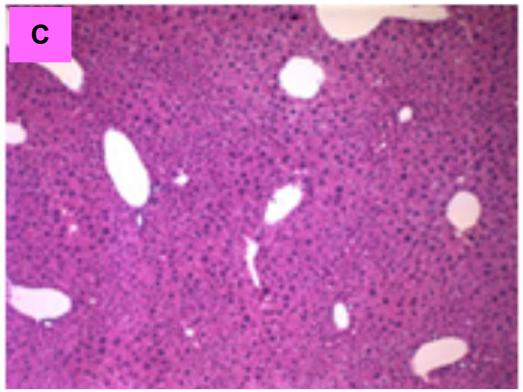
Histological sections of tumors of *neil1*$^{-/-}$, *nth1*$^{-/-}$ and *neil1*$^{-/-}$/*nth1*$^{-/-}$ mice



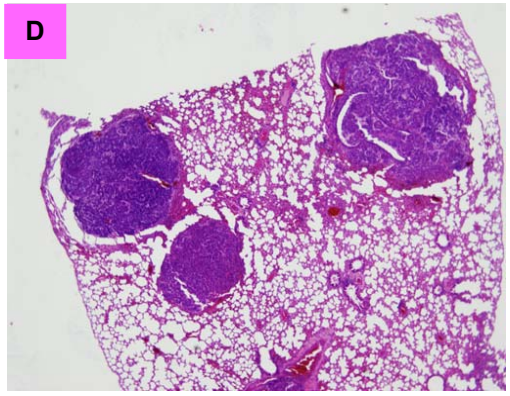
Liver from *neil1*$^{-/-}$ mouse showing nodular hyperplasia of hepatocytes



Liver from *nth1*$^{-/-}$ mouse showing hepatocellular carcinoma



Liver from *neil1*$^{-/-}$/*nth1*$^{-/-}$ mouse showing severe hepatocellular dysplasia, characterized by hyperchromatic and irregularly shaped hepatocyte nuclei



Lung tumors in *neil1*$^{-/-}$/*nth1*$^{-/-}$ mouse with three pulmonary adenocarcinomas

Chan, M. K., Ocampo-Hafalla. M. T., Vartanian, V., Jaruga, P., Kirkali, G., Koenig, K. L., Brown, S., Lloyd, R. S., Dizdaroglu, M. and Teebor, G.W., *DNA Repair* 8, 786-794, 2009

Phenotypes in *neil1* $^{-/-}$ mice

- Severe obesity
- Fatty liver disease
- Hyperinsulinemia
- Dyslipidemia
- Hypertension
- Cancer proneness

  late onset, several types of cancer

Accumulated evidence suggests an important role for NEIL1 in the prevention of the diseases associated with the metabolic syndrome and cancer.

How could the loss of a DNA repair glycosylase lead to this constellation of diseases?

What is the role of FapyAde, FapyGua, R-cdA and S-cdA in disease processes?

DNA repair and cancer therapy

One important mechanism by which cancer cells can develop multi-drug resistance is to increase their DNA repair capacity.

Drugs or radiation kill cancer cells in tumors; however, rapidly growing tumors accumulate mutations that may lead to overexpression of DNA repair genes

Such tumors may be favored by *natural selection* to become capable of surviving and thus develop greater DNA repair capacity than normal tissues.

The toxicity of chemotherapy drugs can be reduced in cancer cells by DNA repair that remove DNA lesions before they become toxic. The efficacy of cancer therapy can thus be influenced by DNA repair capacity.

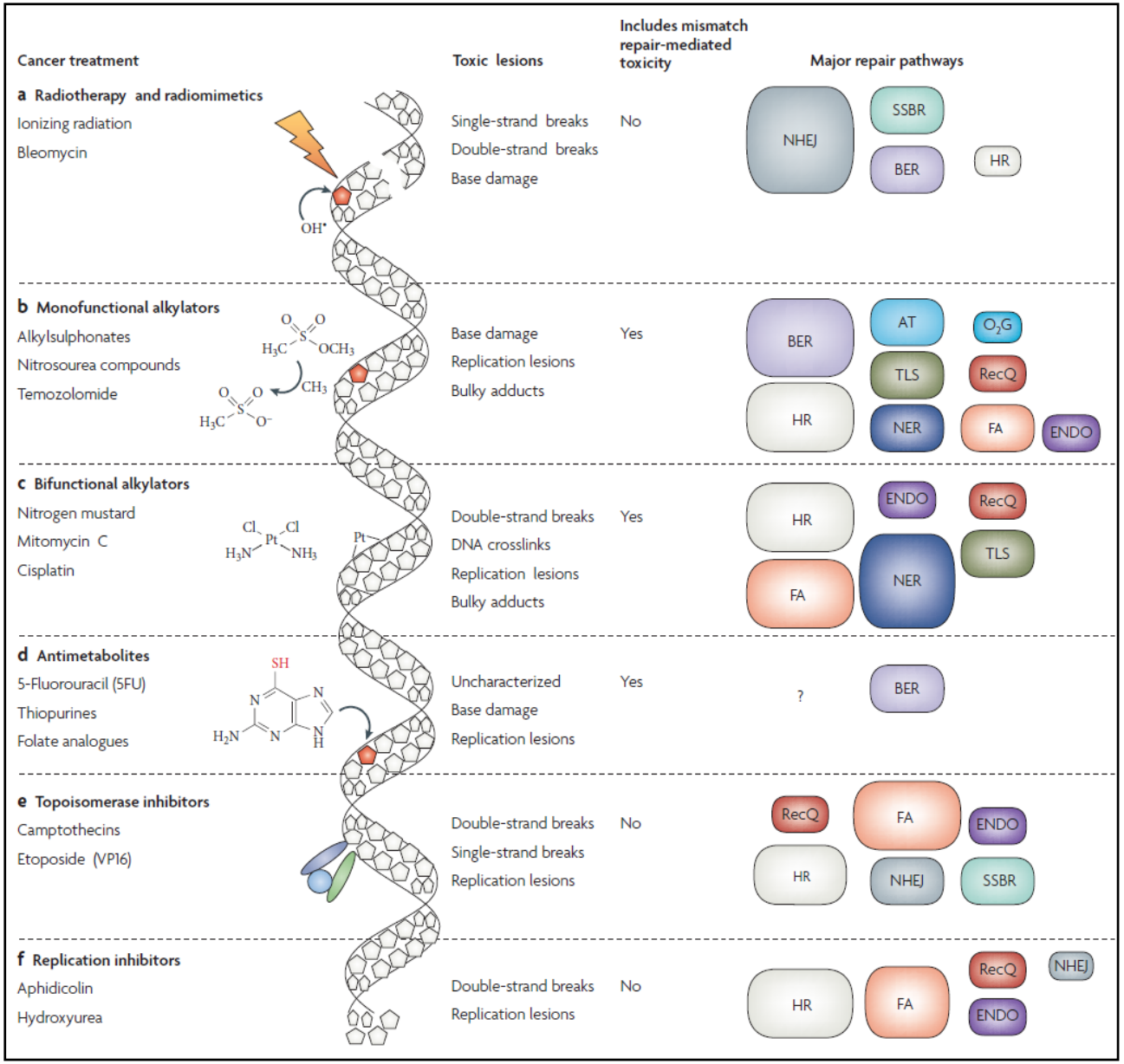
Inhibition of DNA repair

Therefore, DNA repair pathways may be promising targets for novel cancer treatments

DNA repair capacity may a predictor of patient response to cancer therapy.

Reviewed in:
Helleday, T., Petermann, E., Lunding, C., Hodgson, B. and Sharma, R.A., *Nature Reviews Cancer* 8, 193-204, 2008
Kelley, M., *DNA Repair in Cancer Therapy*, Elsevier, 2012

DNA repair pathways that repair DNA lesions formed by cancer therapy



NHEJ: non-homologous end joining

HR: homologous recombination

BER: base excision repair

NER: nucleotide excision repair

SSBR: ss-DNA break repair

ENDO: endonuclease-mediated repair

FA: Fanconie anamia repair

Reviewed in:
Helleday, T., Petermann, E., Lunding, C., Hodgson, B. and Sharma, R.A., *Nature Reviews Cancer* 8, 193-204, 2008

Targeted DNA repair enzymes

Poly (ADP-ribose) polymerase 1 (PARP1)

PARP1 is required for the efficient repair of apurinic/apyrimidic sites (AP site or abasic site), and single-stranded DNA breaks.

It also interacts with BRCA1 and BRCA2.

Cells deficient in BRCA1 or BRCA2 are highly sensitive to PARP1 inhibition.

Apurinic/apyrimidic endonuclease 1 (APE1)

APE1 hydrolyzes the phosphate bond at 5' to the AP site causing a strand breaks.

O^6-methylguanine-DNA methyl transferase (MGMT)

MGMT is an alkyl transferase that removes alkylations on the O^6-position of guanine produced by anticancer agents.

Reviewed in:
Helleday, T., Petermann, E., Lunding, C., Hodgson, B. and Sharma, R.A., *Nature Reviews Cancer* 8, 193-204, 2008
Kelley, M., *DNA Repair in Cancer Therapy*, Elsevier, 2012

Clinical trials of DNA repair inhibitors

Agent (company)	Target molecule or pathway	Monotherapy or combination therapy agent(s)	Phase of clinical trial planned, ongoing or recently completed	Reference
AZD-2281 (Astra Zeneca)	PARP	Gemcitabine Carboplatin Topotecan Monotherapy	Phase I Phase I Phase I Phase II	http://www.astrazenecaclinicaltrials.com/article/525925.aspx
AG014699 (Pfizer)	PARP	Temozolomide antibody Temozolomide	Phase I Phase II	http://www.eddn.org/clinicalTr_caResUK.html
INO-1001 (Inotek)	PARP	Temozolomide	Phase I	http://www.inotekcorp.com/content/ino-1001.asp
BSI-201 (Bipar Sciences)	PARP	Monotherapy Gemcitabine–carboplatin	Phase I Phase II	http://www.biparsciences.com/BSI201.html
ABT-888 (Abbott Laboratories)	PARP	Temozolomide	Phase I	NCT00526617
TRC-102 (Tracon Pharma)	BER	Temozolomide Pemetrexed	Phase I Phase I planned	http://www.traconpharma.com/content/pipeline_overview.html
Lomeguatrib (Astra Zeneca)	MGMT	Irinotecan Temozolomide	Phase I Phase II	http://www.astrazenecaclinicaltrials.com/article/525925.aspx
O^6-Benzylguanine	MGMT	Temozolomide	Phase II	http://clinicalstudies.info.nih.gov/cgi/detail.cgi?A_2006-C-0089.html
Decitabine (MGI Pharma[121])	Hypermethylation of mismatch repair genes	Epirubicin, cisplatin, 5-fluorouracil Carboplatin	Phase I Phase II	http://pfsearch.ukcrn.org.uk/StudyDetail.aspx?TopicID=&StudyID=2192

PARP: poly(ADP-ribose) polymerase
BER: base excision repair
MGMT: O-6-methylguanine-DNA methyl transferase

Reviewed in:
Helleday, T., Petermann, E., Lunding, C., Hodgson, B. and Sharma, R.A., *Nature Reviews Cancer* 8, 193-204, 2008

DNA repair and cancer therapy

"A through understanding of DNA repair pathways dysregulated during carcinogenesis and neoplastic progression, as well as types of DNA damage induced during cancer development, may hold the key to the breakthrough in improving therapy in the fight against cancer."

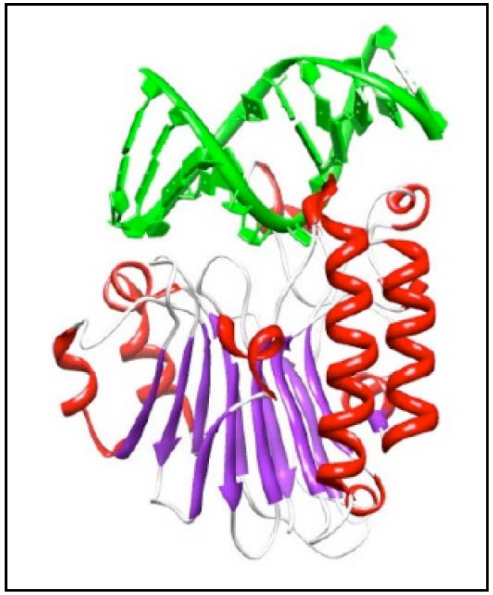
Aziz, K., Nowsheen, S. and Georgakilas, A.G., *Curr. Mol. Med.* 10, 626-639, 2010

"A knowledge of DNA repair proteins' overexpression or underexpression in cancers will help predict and guide development of treatments, and yield the greatest therapeutic response."

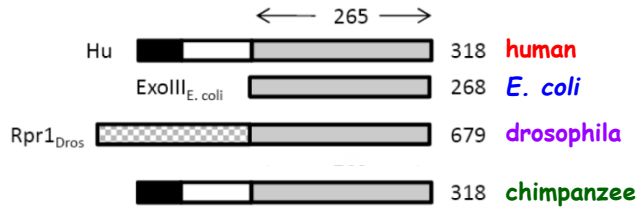
Kelley, M. R., *DNA Repair in Cancer Therapy*, Elsevier, 2012

Mass spectrometric techniques with isotope-dilution are essential for accurate measurement of DNA repair proteins as suitable biomarkers.

Human apurinic/apyrimidinic endonuclease 1 (hAPE1)



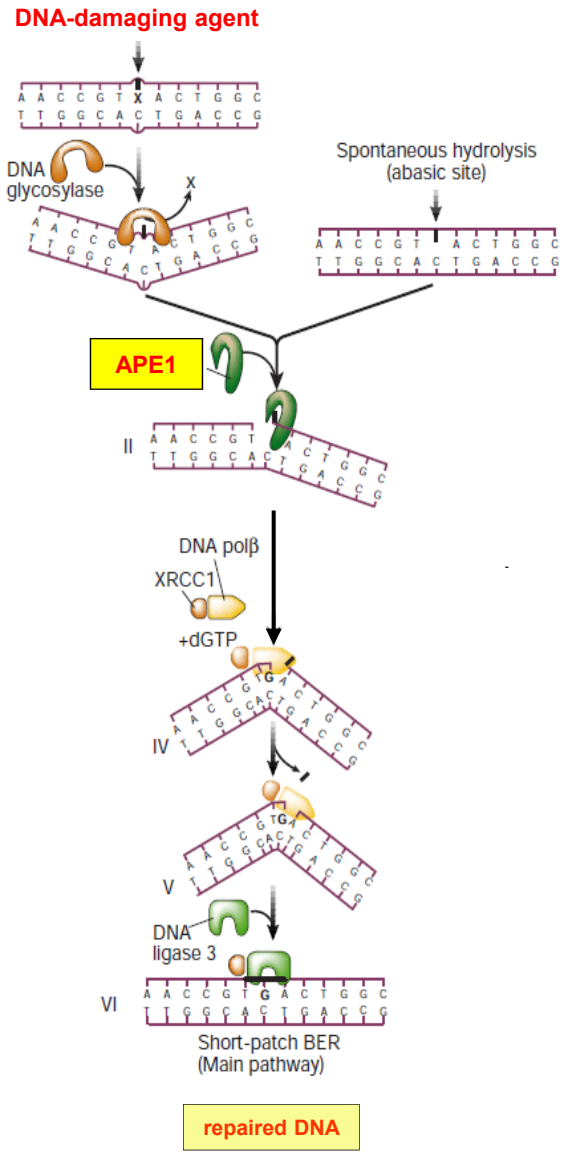
Ribbon diagram of hAPE1

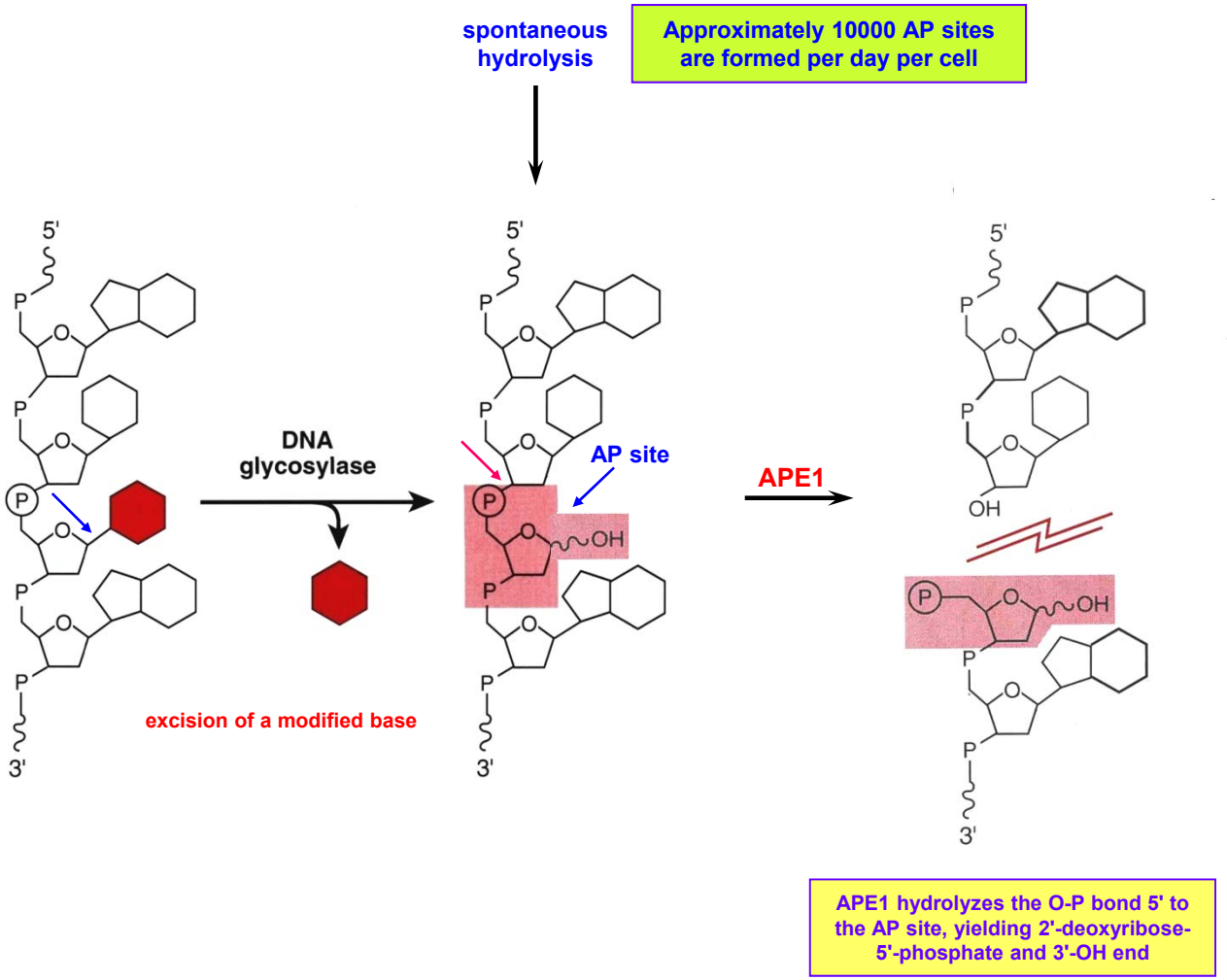



```
        10         20         30         40         50         60
MPKRGKKGAV AEDGDELRTE PEAKKSKTAA KKNDKEAAGE GPALYEDPPD QKTSPSGKPA

        70         80         90        100        110        120
TLKICSWNVD GLRAWIKKKG LDWVKEEAPD ILCLQETKCS ENKLPAELQE LPGLSHQYWS

       130        140        150        160        170        180
APSDKEGYSG VGLLSRQCPL KVSYGIGDEE HDQEGRVIVA EFDSFVLVTA YVPNAGRGLV

       190        200        210        220        230        240
RLEYRQRWDE AFRKFLKGLA SRKPLVLCGD LNVAHEEIDL RNPKGNKKNA GFTPQERQGF

       250        260        270        280        290        300
GELLQAVPLA DSFRHLYPNT PYAYTFWTYM MNARSKNVGW RLDYFLLSHS LLPALCDSKI

       310        318
RSKALGSDHC PITLYLAL
```

Amino acid sequence of hAPE1
Monoisotopic molecular mass, 35532 Da

DNA repair activity of APE1





Reviewed in:
Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A. and Ellenberger, T., *DNA Repair and Mutagenesis*, 2006

Human apurinic/apyrimidinic endonuclease 1 (hAPE1)

DNA repair activity of APE1 is critical for cell viability

1. *Complete absence of APE1 is associated with embryonic lethality in mice*
2. *APE1 depletion hypersensitizes cells to DNA damage*
3. *Overexpression of APE1 protects cells from DNA damage*

APE1 expression is increased in human cancers

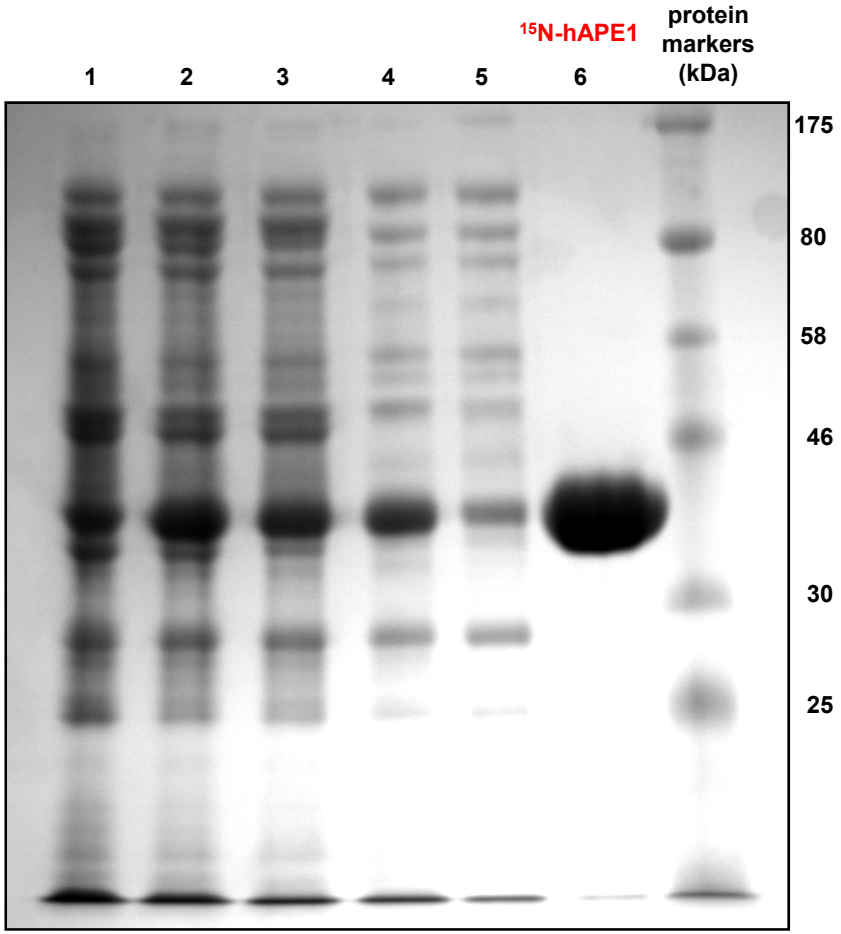
1. *Nuclear and/or cytoplasmic overexpression of APE1 occurs in breast, cervical, colon, head & neck, lung, melanoma, ovarian, prostate, etc., cancers*
2. *Increased APE1 expression is associated with resistance to chemo- and radiation therapies*
3. *Alterations in subcellular distribution of APE1 compared to normal tissues have been observed in a number of human tumors*

APE1 as a predictive and prognostic biomarker

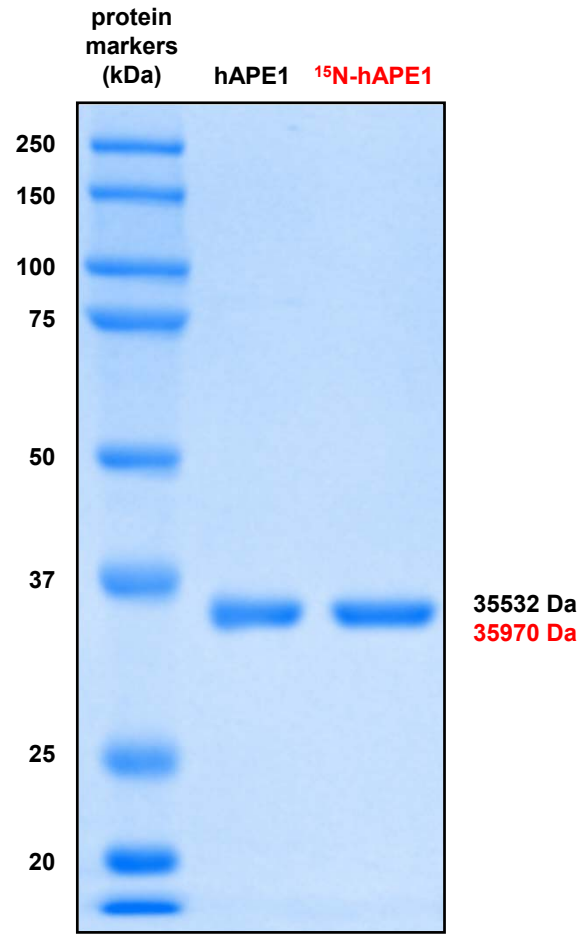
1. *Nuclear localization is associated with good prognostic features*
2. *Cytoplasmic localization is associated with poor survival outcomes*

Positive identification and accurate quantification of hAPE1 in tissues is essential for its use as an efficient biomarker

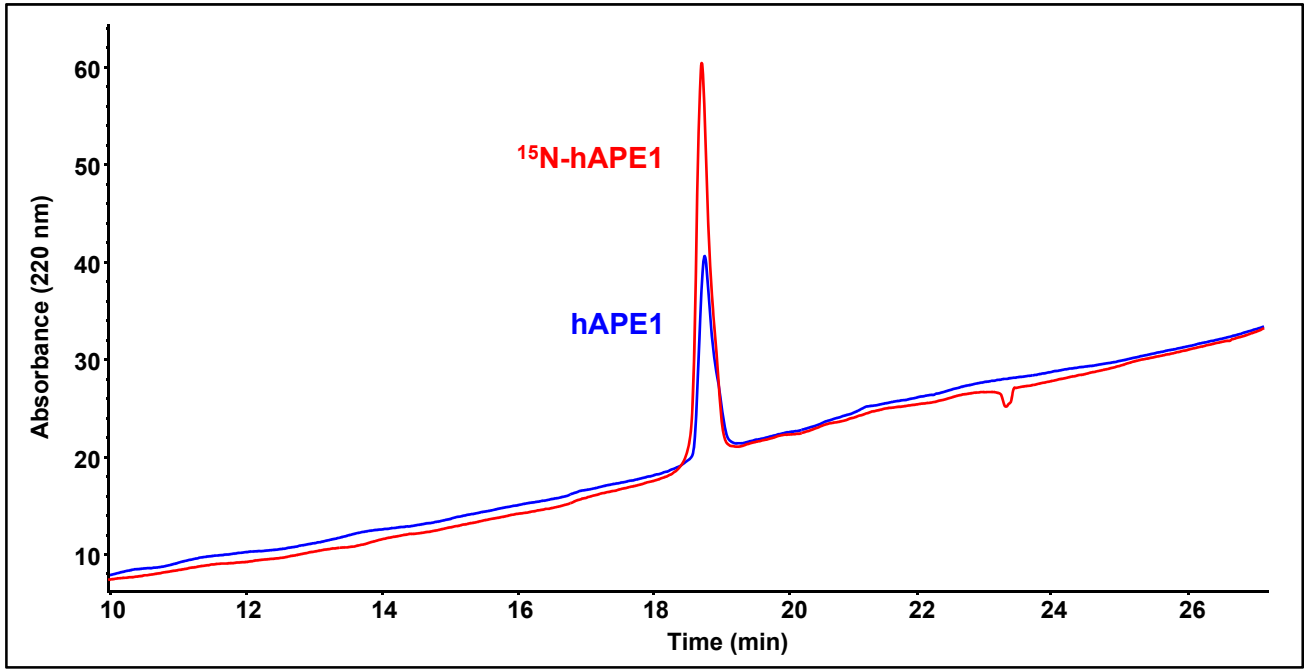
Purification of ^{15}N-hAPE1



Lane 1: Uninduced cell extract
Lane 2: Induced cell extract
Lane 3: 70,000 x g supernatant fraction
Lane 4: Flow through from DEAE cellulose column
Lane 5: Flow through from CM cellulose column
Lane 6: Purified ^{15}N-hAPE1



Separation of hAPE1 and ^{15}N-hAPE1 by HPLC



Peptides resulting from trypsin hydrolysis of hAPE1

```
        10         20         30         40         50         60
MPKRGKKGAV AEDGDELRTE PEAKKSKTAA KKNDKEAAGE GPALYEDPPD QKTSPSGKPA

        70         80         90        100        110        120
TLKICSWNVD GLRAWIKKKG LDWVKEEAPD ILCLQETKCS ENKLPAELQE LPGLSHQYWS

       130        140        150        160        170        180
APSDKEGYSG VGLLSRQCPL KVSYGIGDEE HDQEGRVIVA EFDSFVLVTA YVPNAGRGLV

       190        200        210        220        230        240
RLEYRQRWDE AFRKFLKGLA SRKPLVLCGD LNVAHEEIDL RNPKGNKKNA GFTPQERQGF

       250        260        270        280        290        300
GELLQAVPLA DSFRHLYPNT PYAYTFWTYM MNARSKNVGW RLDYFLLSHS LLPALCDSKI

       310        318
RSKALGSDHC PITLYLAL
```

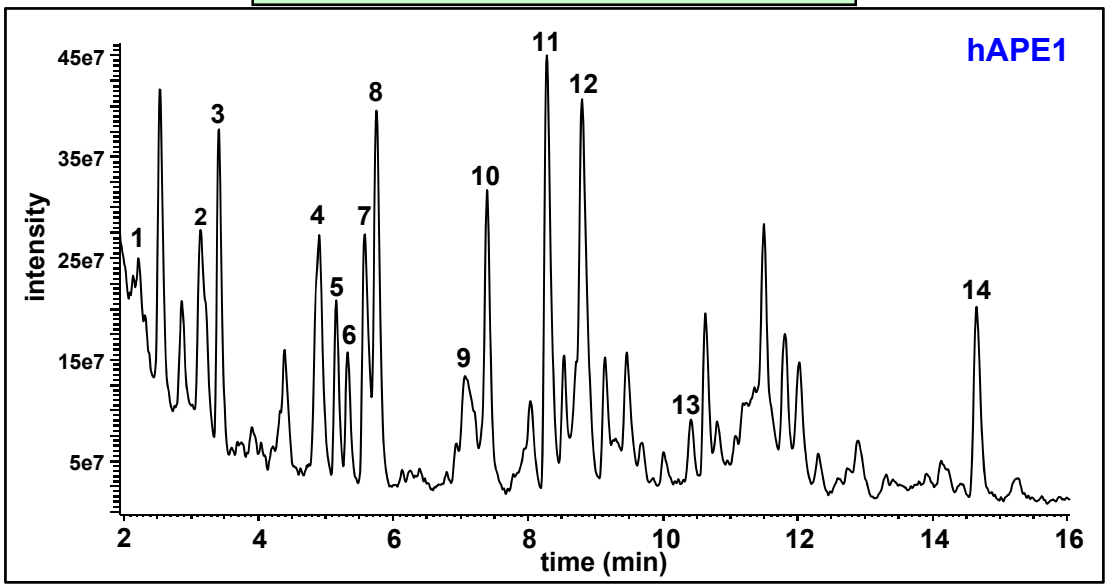
Position of cleavage site	Name of cleaving enzyme(s)	Resulting peptide sequence (see explanations)	Peptide length [aa]	Peptide mass [Da]
3	Trypsin	MPK	3	374.499
4	Trypsin	R	1	174.203
6	Trypsin	GK	2	203.241
7	Trypsin	K	1	146.189
18	Trypsin	GAVAEDGDELR	11	1131.164
24	Trypsin	TEPEAK	6	673.721
25	Trypsin	K	1	146.189
27	Trypsin	SK	2	233.268
31	Trypsin	TAAK	4	389.452
32	Trypsin	K	1	146.189
35	Trypsin	NDK	3	375.382
52	Trypsin	EAAGEGPALYEDPPDQK	17	1786.869
63	Trypsin	TSPSGKPATLK	11	1086.254
73	Trypsin	ICSWNVDGLR	10	1162.329
77	Trypsin	AWIK	4	516.641
78	Trypsin	K	1	146.189
79	Trypsin	K	1	146.189
85	Trypsin	GLDWVK	6	716.835
98	Trypsin	EEAPDILCLQETK	13	1488.673
103	Trypsin	CSENK	5	579.626
125	Trypsin	LPAELQELPGLSHQYWSAPSDK	22	2466.732
136	Trypsin	EGYSGVGLLSR	11	1137.258
141	Trypsin	QCPLK	5	587.735
156	Trypsin	VSYGIGDEEHDQEGR	15	1690.700
177	Trypsin	VIVAEFDSFVLVTAYVPNAGR	21	2267.610
181	Trypsin	GLVR	4	443.547
185	Trypsin	LEYR	4	579.654
187	Trypsin	QR	2	302.333
193	Trypsin	WDEAFR	6	822.875
194	Trypsin	K	1	146.189
197	Trypsin	FLK	3	406.525
202	Trypsin	GLASR	5	502.571
221	Trypsin	KPLVLCGDLNVAHEEIDLR	19	2134.478
224	Trypsin	NPK	3	357.410
227	Trypsin	GNK	3	317.345
228	Trypsin	K	1	146.189
237	Trypsin	NAGFTPQER	9	1019.082
254	Trypsin	QGFGELLQAVPLADSFR	17	1848.089
274	Trypsin	HLYPNTPYAYTFWTYMMNAR	20	2540.896
276	Trypsin	SK	2	233.268
281	Trypsin	NVGWR	5	630.704
299	Trypsin	LDYFLLSHSLLPALCDSK	18	2035.386
301	Trypsin	IR	2	287.362
303	Trypsin	SK	2	233.268
318	**end of sequence**	ALGSDHCPITLYLAL	15	1586.866

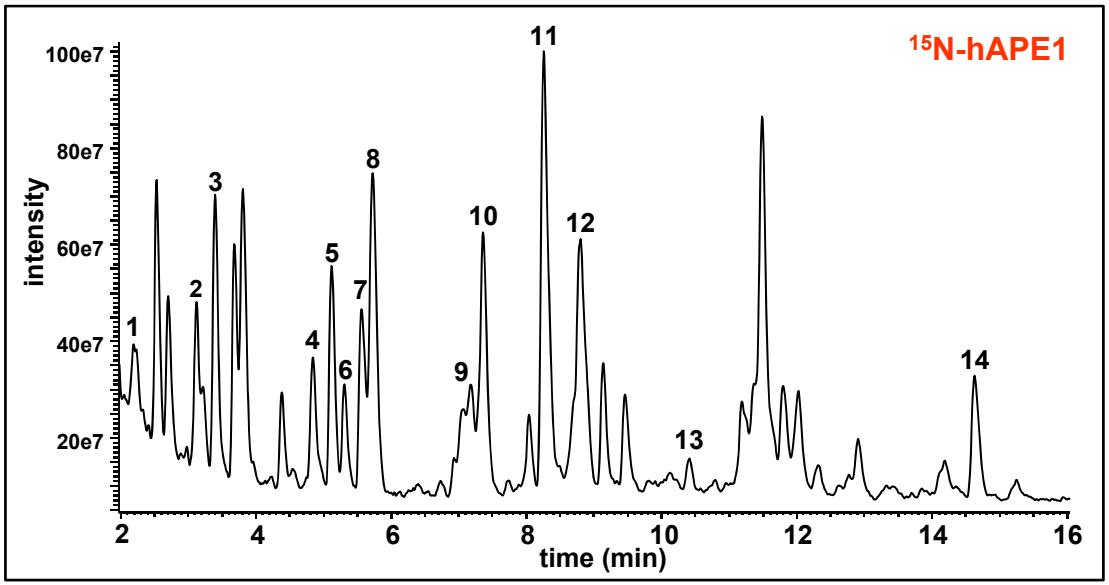
http://web.expasy.org/cgi-bin/peptide_cutter/peptidecutter.pl

37 tryptic peptides, 1 arginine, 7 lysines

Measurement of hAPE1 by LC-MS/MS with isotope-dilution

Total-ion-current profiles of tryptic peptides





Sequences of the identified peptides and the *m/z* values of their MH^+ and $(M+2H)^{2+}$ ions

		unlabeled peptide		^{15}N-labeled peptide	
peak	peptide	MH^+	$(M+2H)^{2+}$	MH^+	$(M+2H)^{2+}$
1	GLASR	503.2	252.1	511.2	256.1
2	TSPSGKPATLK	1086.6	543.8	1099.6	550.3
3	GLVR	444.2	222.6	452.2	226.6
4	NAGFTPQER	1019.4	510.2	1033.4	517.2
5	NVGWR	631.3	316.1	641.3	321.1
6	GAVAEDGDELR	1131.5	566.2	1145.5	573.2
7	VSYGIGDEEHDQEGR	1690.7	845.8	1711.7	856.3
8	AWIK	517.3	259.1	523.3	262.1
9	EAAGEGPALYEDPPDQK	1786.8	893.9	1805.8	903.4
10	WDEAFR	823.3	412.1	833.3	417.1
11	GLDWVK	717.3	359.1	725.3	363.1
12	EGYSGVGLLSR	1137.5	569.2	1151.5	576.2
13	EEAPDILCLQETK	1488.7	744.8	1503.7	752.3
14	QGFGELLQAVPLADSFR	1847.9	924.4	1869.9	935.4

Protein score obtained = 150
[protein score > 56, statistically significant ($p < 0.05$)]

Identification of proteins by LC-MS/MS

1. Hydrolysis of protein with trypsin

H_2N–(…X–X…)–Arg┆(…X–X…)–COOH H_2N–(…X–X…)–Lys┆(…X–X…)–COOH

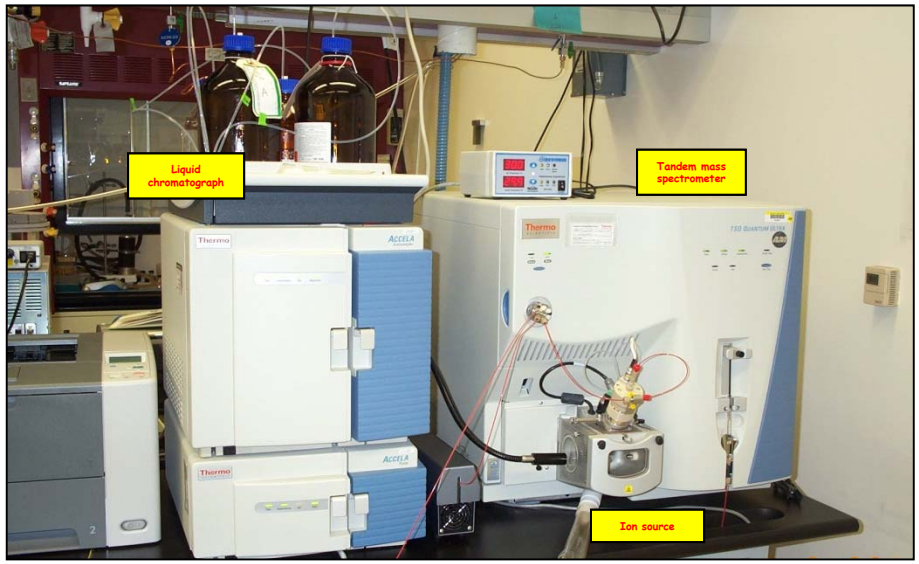
2. Separation of the resulting tryptic peptides by LC

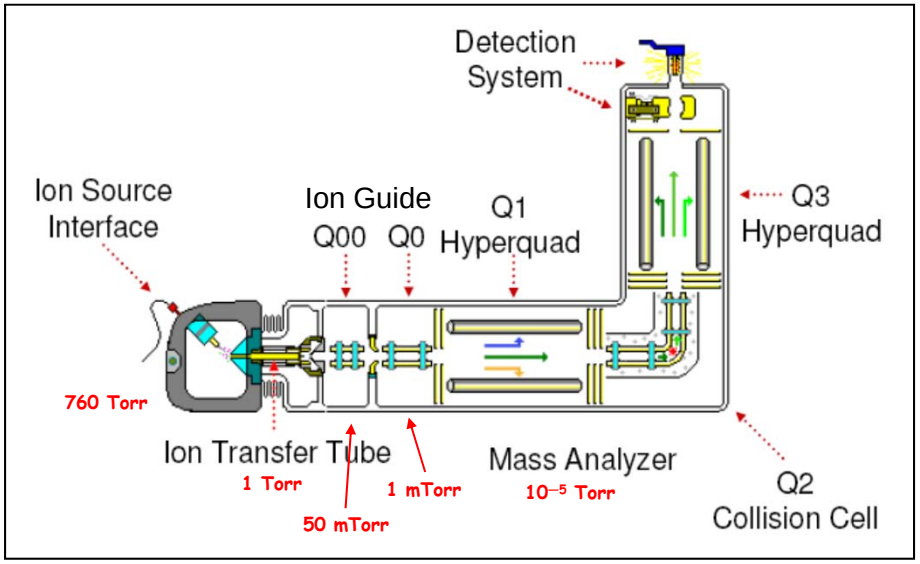
3. Recording total ion-current profiles and full scan mass spectra of separated tryptic peptides using the first mass spectrometer (Q1)

4. Fragmentation of peptides in the collision cell (Q2)

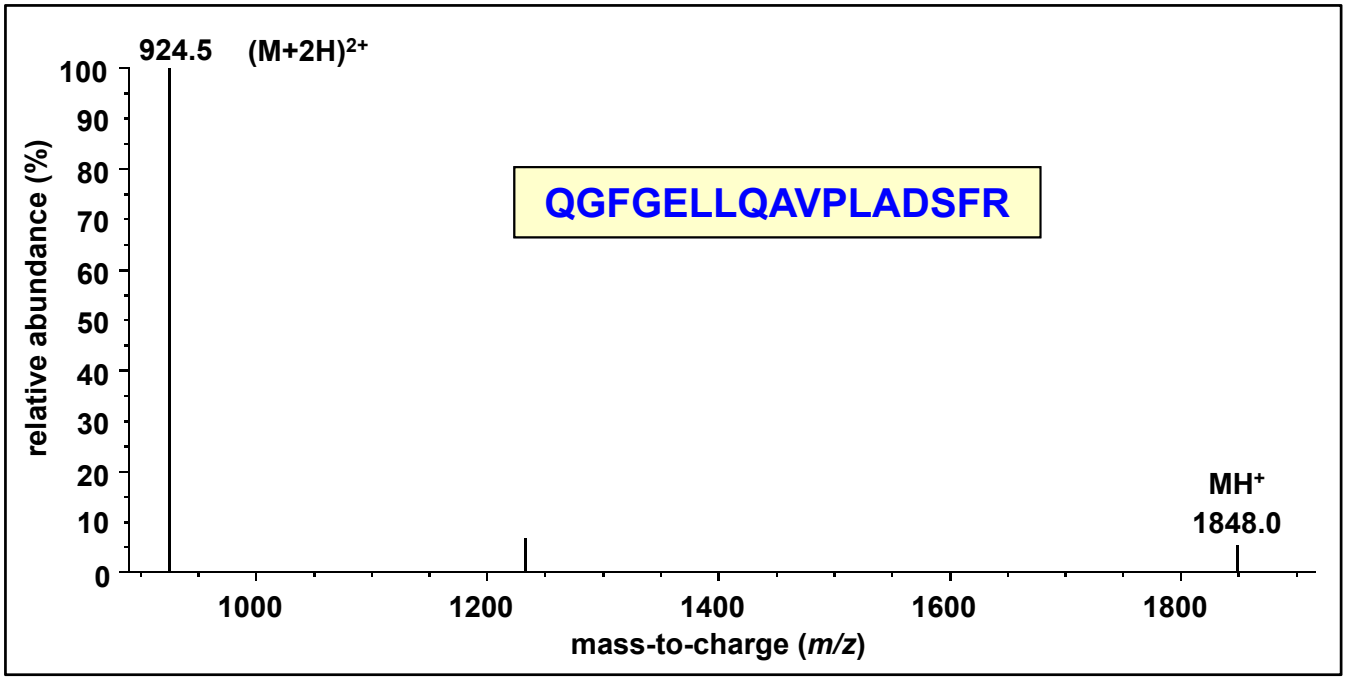
5. Recording the product ion spectrum of each peptide in the second mass spectrometer (Q3)

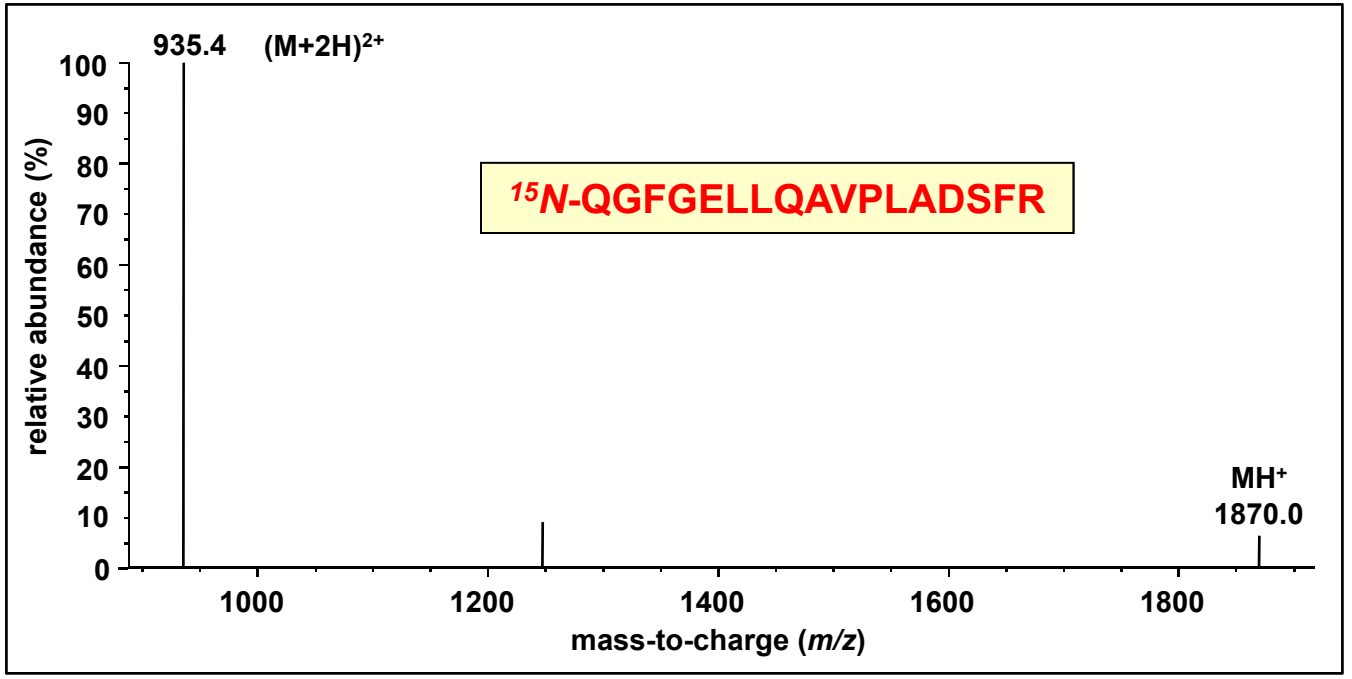
6. Multiple reaction monitoring of the fragment ions in the product ion spectra (Q3)



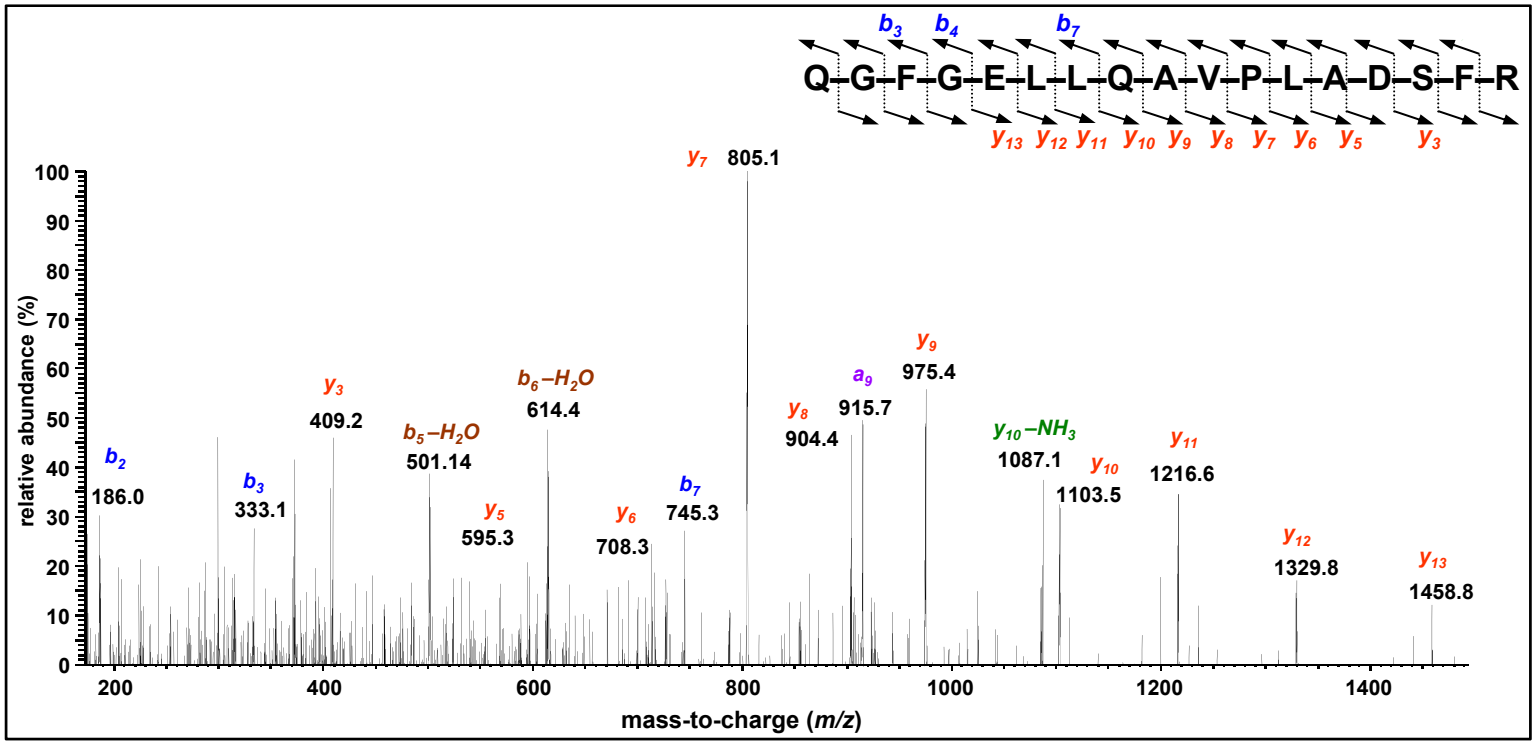


Full scan-mass spectrum of a tryptic peptide of hAPE1 and its ^{15}N-labeled analogue





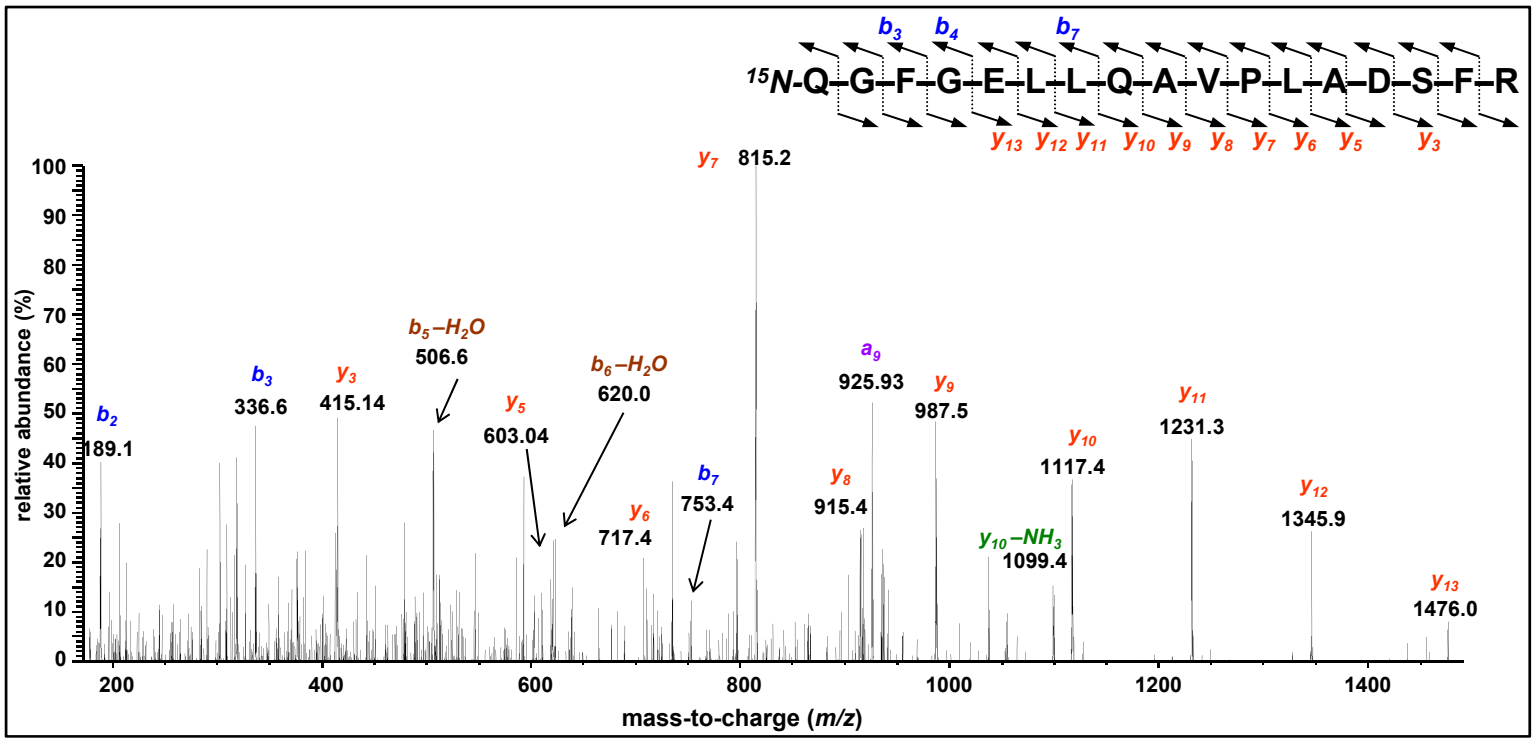
Product ion spectra of a tryptic peptide of hAPE1 and its ^{15}N-labeled analogue



MH$^+$ 1848.0 Da
(M + 2H)$^{2+}$ 924.5 Da

Mass transitions

m/z 924.5 → *m/z* 805.4
m/z 924.5 → *m/z* 904.5
m/z 924.5 → *m/z* 975.5
m/z 924.5 → *m/z* 1103.6
m/z 924.5 → *m/z* 1216.7
m/z 924.5 → *m/z* 1329.8
m/z 924.5 → *m/z* 1458.8

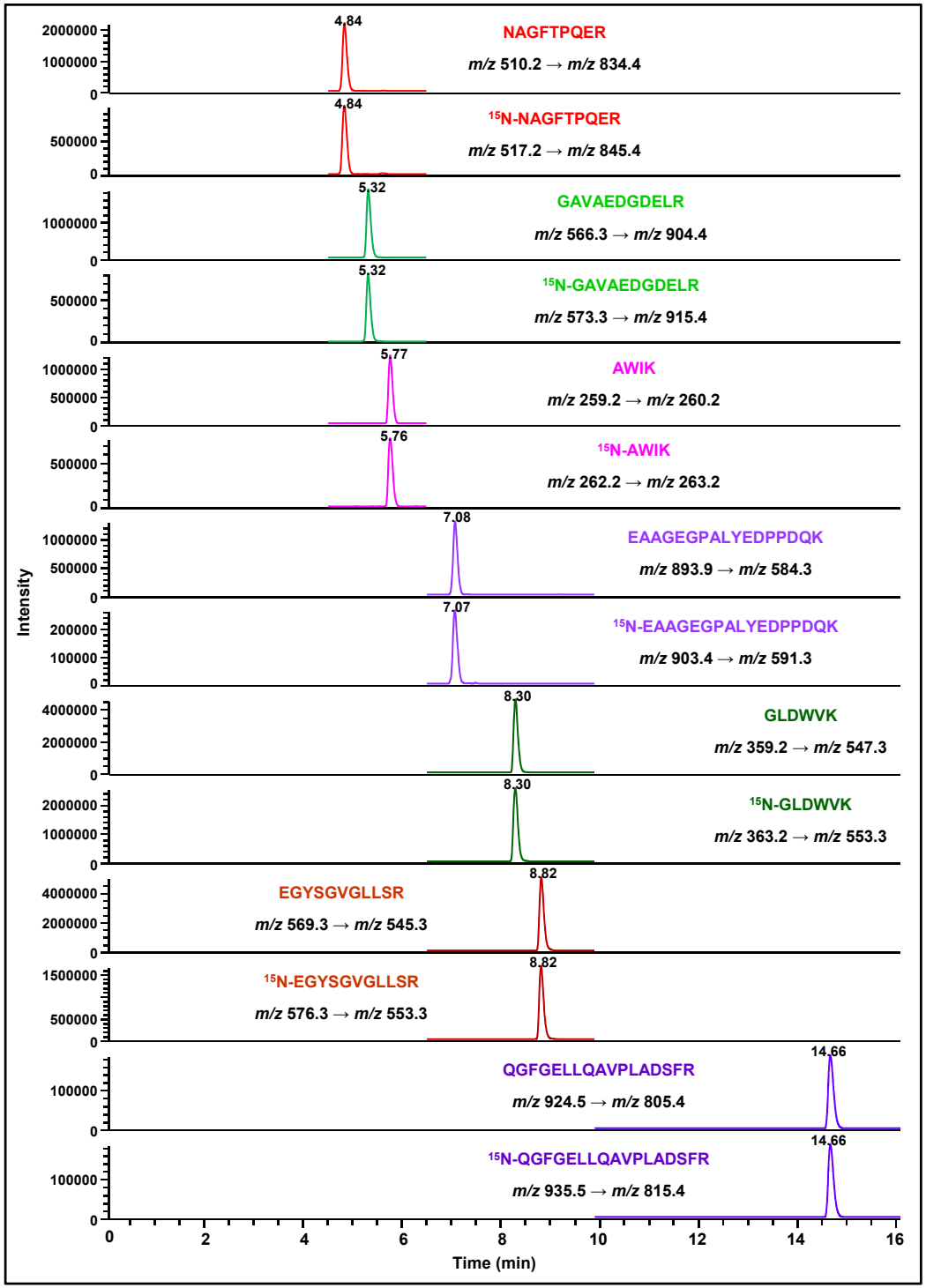


MH$^+$ 1870.0 Da
(M + 2H)$^{2+}$ 935.5 Da

Mass transitions

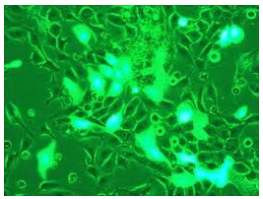
m/z 935.5 → *m/z* 815.4
m/z 935.5 → *m/z* 915.5
m/z 935.5 → *m/z* 987.5
m/z 935.5 → *m/z* 1117.6
m/z 935.5 → *m/z* 1231.7
m/z 935.5 → *m/z* 1345.8
m/z 935.5 → *m/z* 1475.8

Multiple reaction monitoring of tryptic peptides of hAPE1 and their ^{15}N-labeled analogues

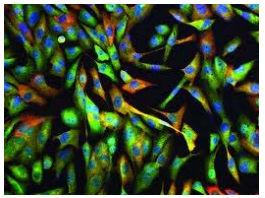


Quantification of APE1 in human cells and mouse liver

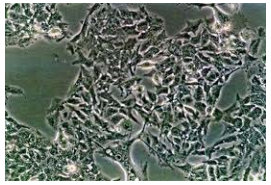
MCF-10A: mammary gland epithelial cell line



MCF-7: mammary gland epithelial adenocarcinoma cell line

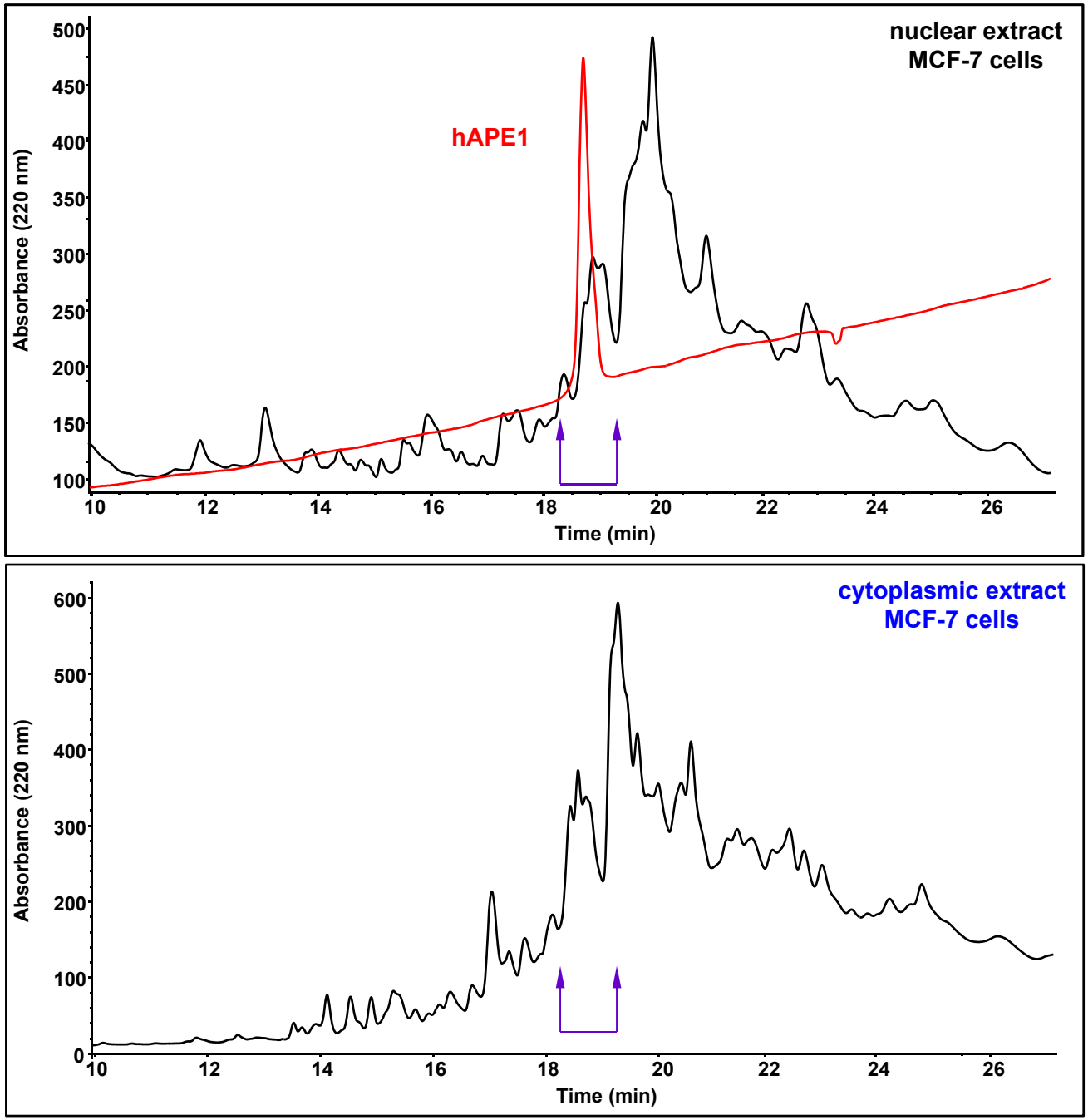


HepG-2: hepatocellular carcinoma cell line

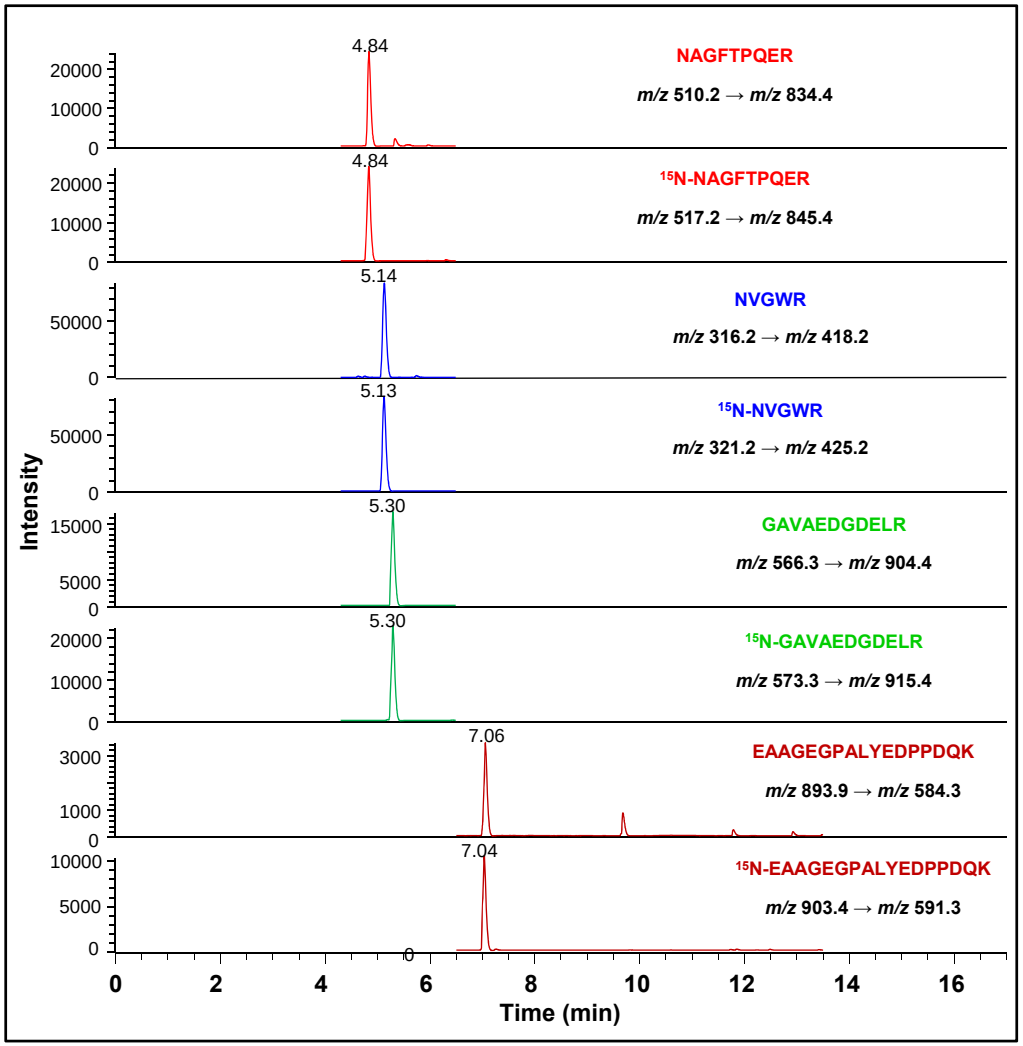


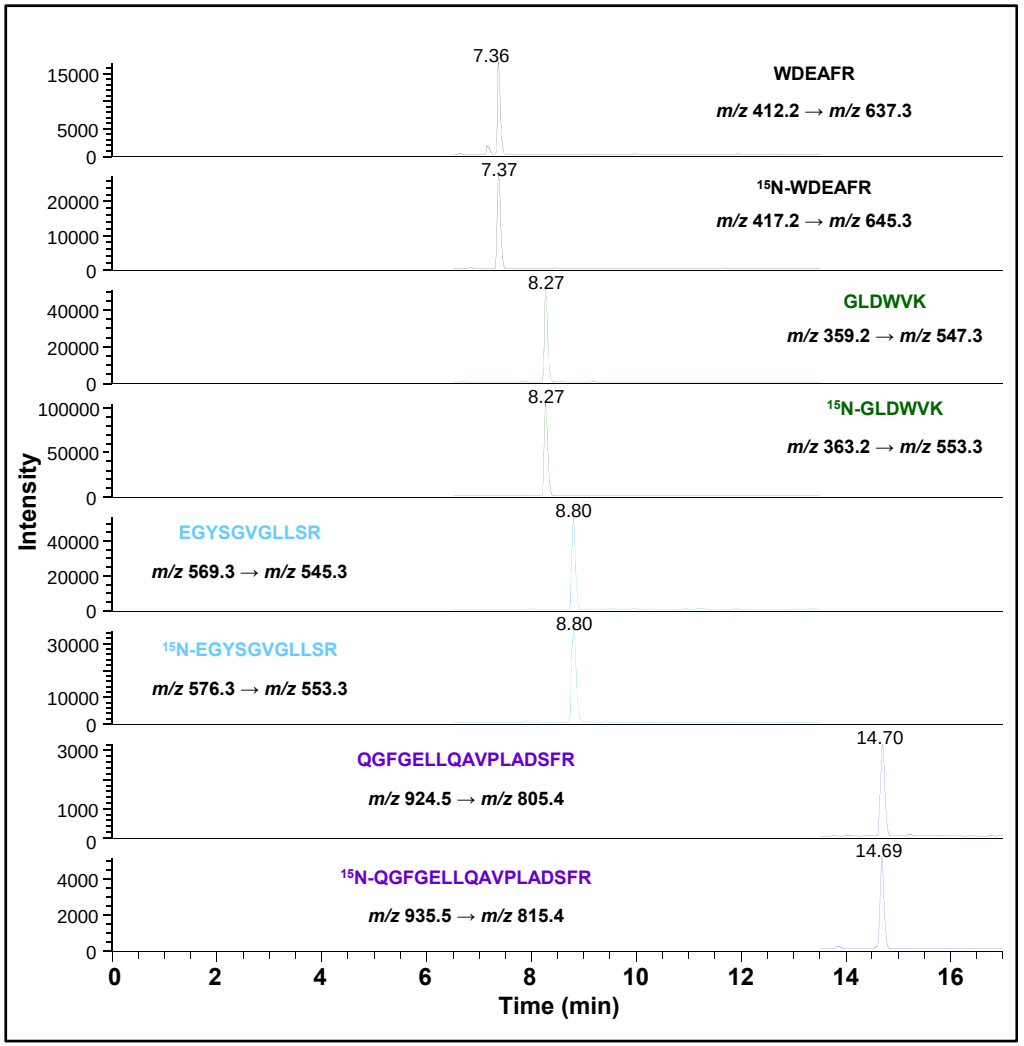
mouse liver

Enrichment of hAPE1 by HPLC from protein extracts of human cells



Identification of hAPE1 in human MCF-10A cells

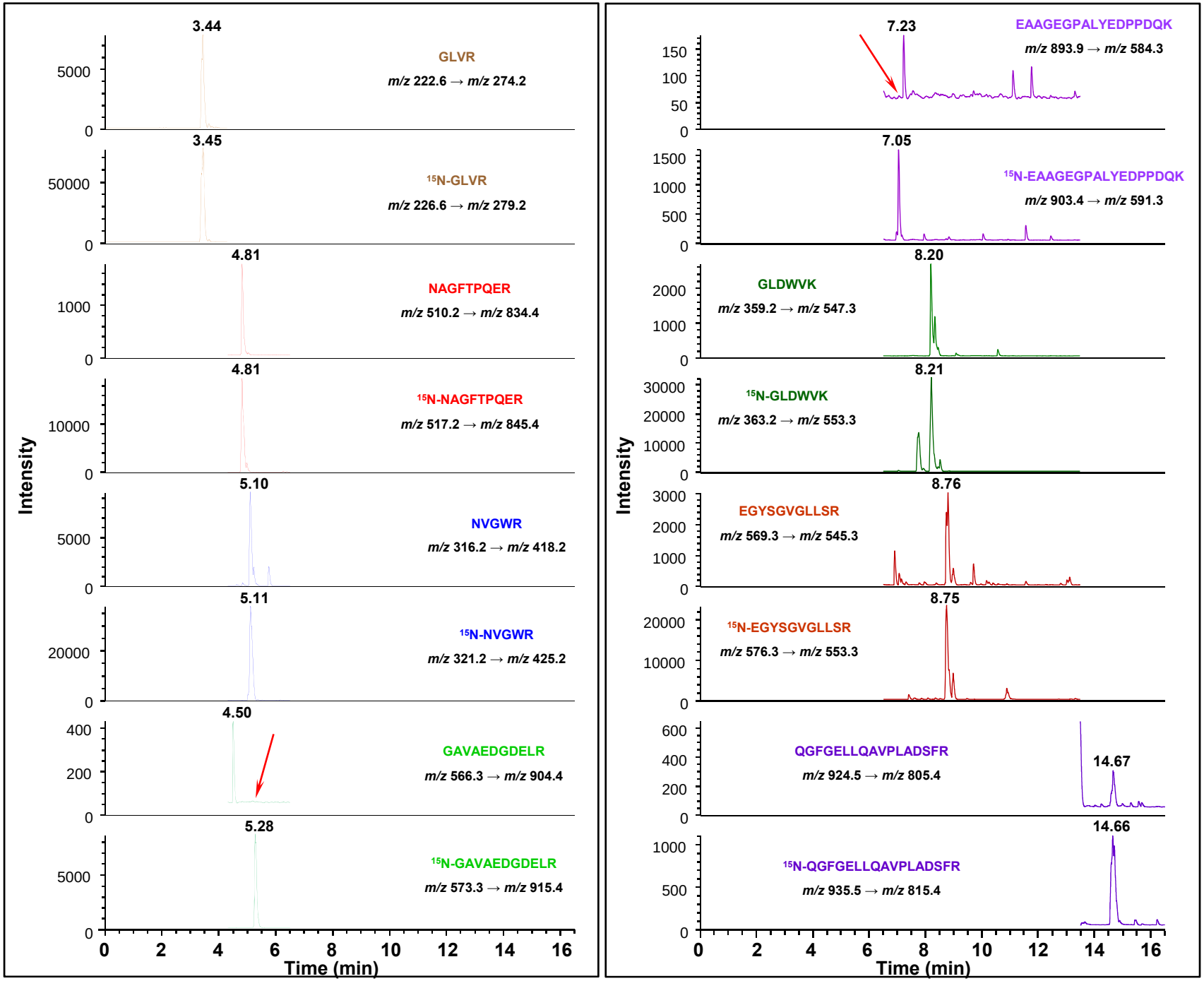




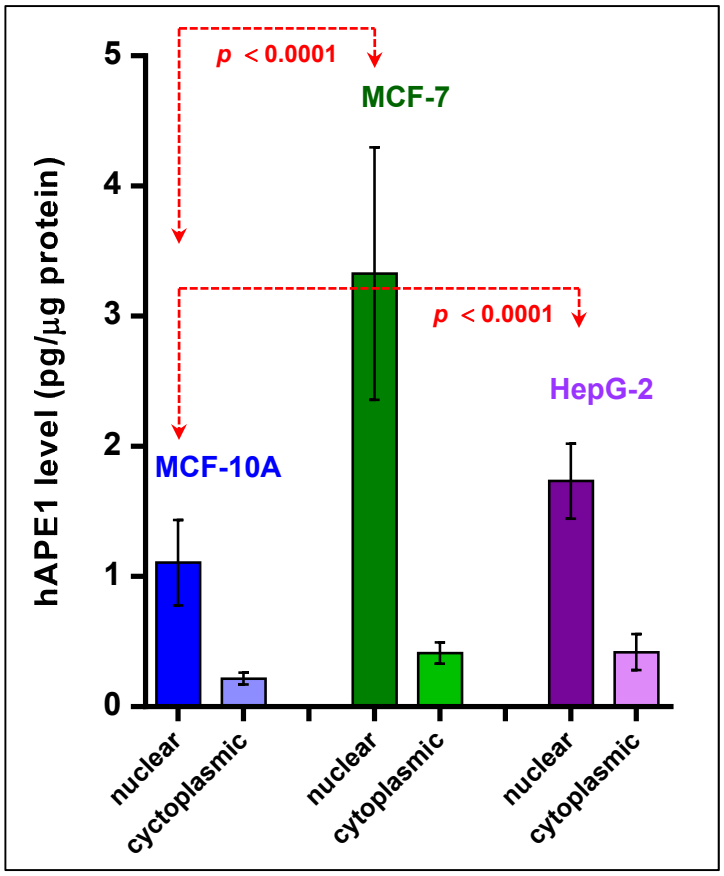
Comparison of the tryptic peptides of human APE1 and mouse APE1

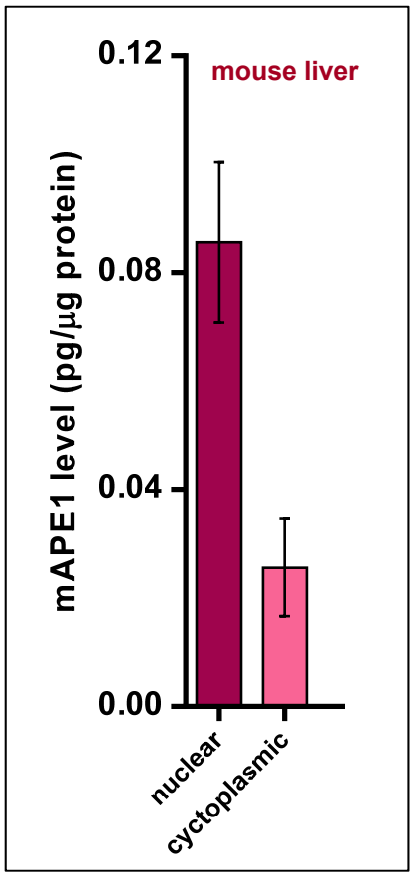
human peptides	mouse peptides
GLASR	
TSPSGKPATLK	
GLVR	GLVR
NAGFTPQER	NAGFTPQER
NVGWR	NVGWR
GAVAEDGDELR	
VSYGIGDEEHDQEGR	
AWIK	AWIK
EAAGEGPALYEDPPDQK	
WDEAFR	WDEAFR
GLDWVK	GLDWVK
EGYSGVGLLSR	EGYSGVGLLSR
EEAPDILCLQETK	
QGFGELLQAVPLADSFR	QGFGELLQAVPLADSFR

Identification of hAPE1 in mouse liver



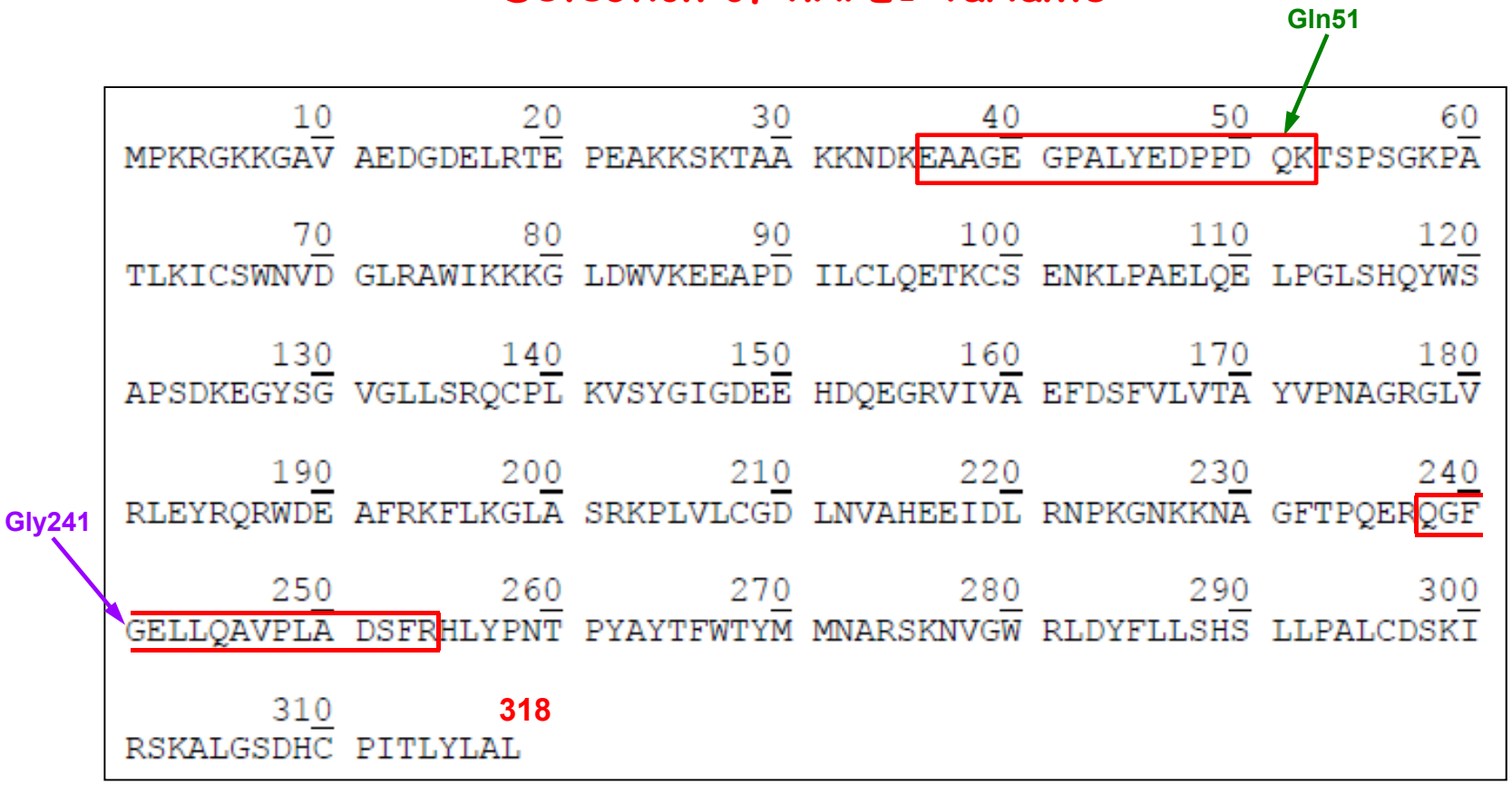
Quantification of APE1 in human cells and mouse liver





MCF-10A: mammary gland epithelial cell line
MCF-7: mammary gland epithelial adenocarcinoma cell line
HepG-2: hepatocellular carcinoma cell line

Detection of hAPE1 variants



Gln51His variant

EAAGEGPALYEDPPDQK

MH^+ *m/z* 1786.8
$(M+2H)^{2+}$ *m/z* 893.9

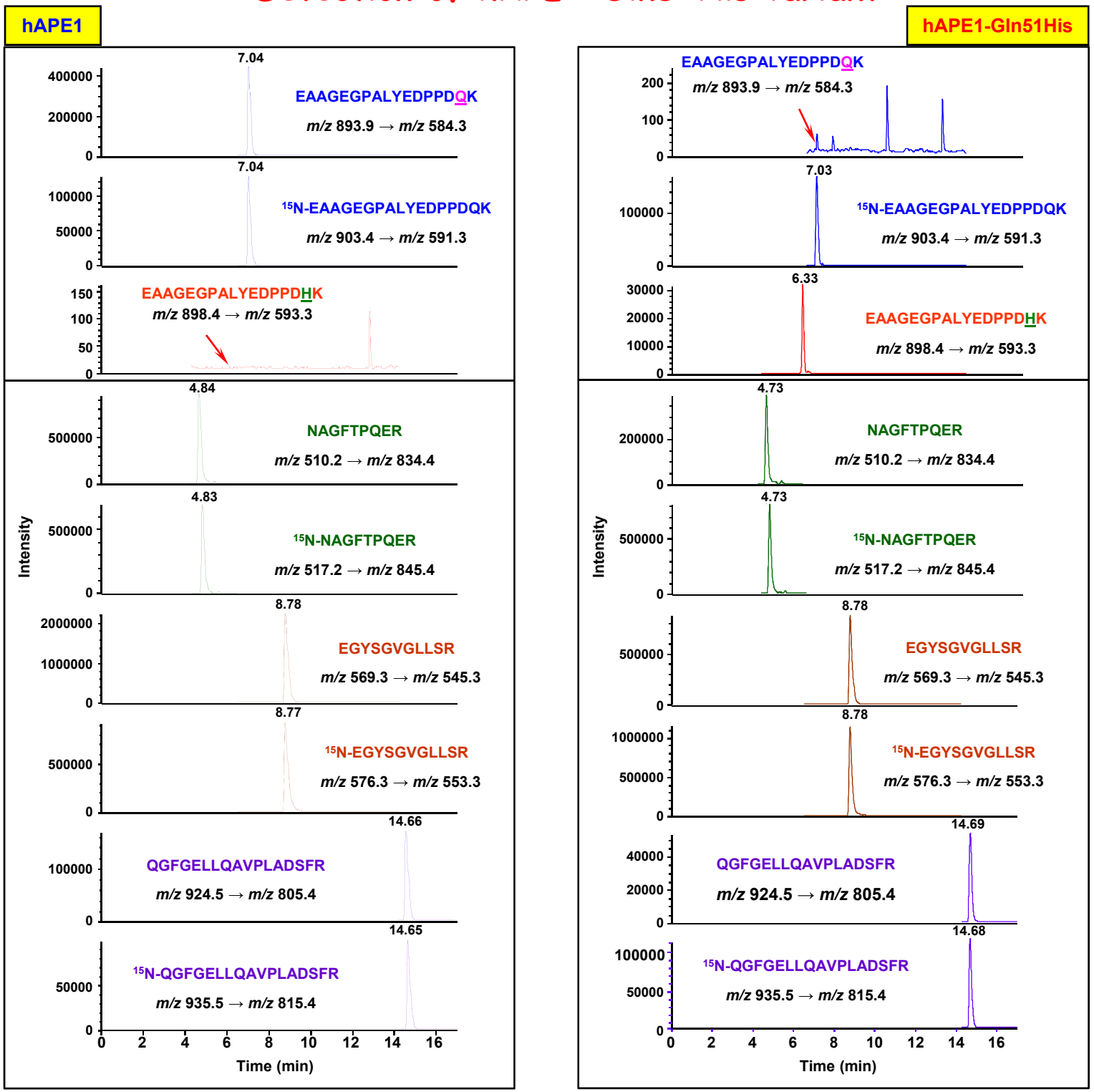
m/z 893.9 → *m/z* 584.3
y_5 - ion

EAAGEGPALYEDPPDHK

MH^+ *m/z* 1795.8
$(M+2H)^{2+}$ *m/z* 898.4

m/z 898.4 → *m/z* 593.3
y_5 -ion

Detection of hAPE1-Gln51His variant

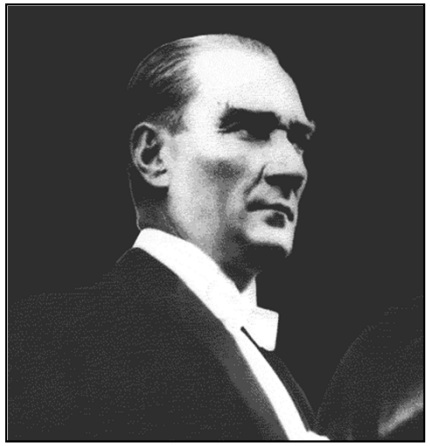


Collaborators

Pawel Jaruga, NIST
Güldal Kirkali, NIST
Bryant C. Nelson, NIST
Prasad T. Reddy, NIST
Gamze Tuna, NIST

Vilhelm A. Bohr, USA
Serge Boiteux, France
Philip J. Brooks, USA
Mustafa Birincioglu, Turkey
Marcus Cooke, UK
Walter Deutsch, USA
Paul Doetsch, USA
Eugenia Dogliotti, Italy
Kent Gates, USA
Arthur Grollman, USA
Barry Halliwell, Singapore
Bensu Karahalil, Turkey

Asuman Karakaya, Turkey
Zeliha Kerry, Turkey
Jacques Laval, France
Stephen Lloyd, USA
Donald Malins, USA
Sankar Mitra, USA
Ryszard Olinski, Poland
Pablo Radicella, France
Teresa Roldan-Arjona, Spain
George Teebor, USA
Susan S. Wallace, USA
David M. Wilson III, USA



"Dünyada her şey için, medeniyet için, yaşam için, başarı için en gerçek yol gösterici ilimdir, fendir. İlim ve fennin dışında yol gösterici aramak gaflettir, cehalettir, doğru yoldan sapmaktır."

Gazi Mustafa Kemal Atatürk
1881-1938

Teşekkürler

miral@nist.gov
http://www.nist.gov/mml/bmd/miral_dizdar.cfm