Journal Name

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

Dynamic Article Links ►

ARTICLE TYPE

Supplementary Material

Anti-cancer barrier in tumor evolution: Differential expression analysis of genome maintenance pathways and genes

Éder Maiquel Simão,[a] Marialva Sinigaglia ,[b] Cristhian Augusto Bugs,[ac] Mauro Ântonio Alves Castro,[d] Giovani Rubert Librelotto,[e] Ronnie Alves,[f] and José Carolos Merino Mombach*[a]

Discussion of all differentially expressed genes found in the DEG analysis

In Fig. 2 we present six Venn diagrams of the consensus of differentially expressed genes between, at least, two adenomas or cancers for each pathway. In what follows, we give a detailed description of each gene function. The description of subpathways and tissues with the genes altered in consensus is also available in the Supplementary material. The references to the functions of some proteins of consensus can also be found in the database: http://www.ncbi.nlm.nih.gov.

Consensus of genes involved in DDR pathway - adenomas

In Fig. 2a we present the altered genes in the DDR pathway between colon, adrenocortical, pancreatic and thyroid adenomas:

MSH2, MSH3 and MSH6: These genes are components of the post-replicative DNA mismatch repair system (MMR). They are involved in the Single-strand Break DNA repair by Base excision repair and Mismatch repair.

ATR: Is a kinase protein which activates checkpoint signaling upon genotoxic stresses such as ionizing radiation (IR), ultraviolet light (UV) or DNA replication stalling, thereby acting as a DNA damage sensor. It actives the proteins: BRCA1, CHEK1, MDM2, RAD17, RPA2, SMC1 and TP53, which collectively inhibit DNA replication and mitosis and promote DNA repair, recombination and apoptosis. Phosphorylates by H2AFX at sites of DNA damage thereby, regulating DNA damage[1].

ATM: ataxia telangiectasia mutated; Serine/threonine kinase protein which activates checkpoint signaling upon double-strand breaks (DSB), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA), thereby acting as a DNA damage sensor. Phosphorylates by H2AFX at DSB thereby, regulating DNA damage response mechanism. When involved in signal transduction and cell cycle control, it may function as a tumor suppressor[1].

FANCD2: Required for maintenance of chromosomal stability. It promotes accurate and efficient pairing of homologous during meiosis. It is involved in the repair of DNA double-strand breaks by homologous recombination. It promotes the activation of the BRCA2/FANCD1.

MRE11A: Is a protein of the MRN protein complex that plays a central role in the repair of DSBs. This protein is a sensor of damage responsible for facilitating the activation of other proteins including ATM and ATR[1].

RAD52: Involved in Double-stranded break repair. It plays a central role in genetic recombination and DNA repair by promoting the repair of complementary single-stranded DNA and by the RAD51 stimulation.

RAD50: Component of the MRN complex, which plays a central role in DSB repair, DNA recombination, maintenance of telomere integrity and meiosis. The complex possesses single-strand endonuclease activity and double-strand specific 3'-5' exonuclease activity, which are provided by MRE11A. RAD50 may be required to bind DNA ends and hold them in close proximity.

PRKDCA: Kinase Protein, DNA activated, catalytic polypeptide; kinase Serine/threonine-protein that acts as a molecular sensor for DNA damage. It is involved in DNA non homologous end joining (NHEJ) required for DSB repair.

DCLRE1C: DNA cross-link repair 1C. This gene encodes a nuclear protein that is involved in somatic recombination and DNA repair. It has the alloys for the HYRC protein which is involved in DNA non homologous end joining (NHEJ) also required for DSB repair.

BRCA2: Tumor suppressor involved in the repair of damaged chromosomes and with an important role in repairing errors caused by DSB[2].

XRCC4 and LIG4: Form a complex responsible for the binding steps of NHEJ and XRCC4 and increases the adhesion activity of LIG4[3].

RFC3: Acts in Mismatch Repair, Nucleotide Excision Repair and cell cycle checkpoints. The gene interacts with other genes of the family replication factor C.

TP53BP1: The signaling of ATM and ATR substrates is dependent on TP53BP1 that regulates the interactions between the MRN complex (MRE11A) and the two kinases (Schultz et al. 2000). TP53BP1 also stimulates the activation of the Non-homologous end joining repair subpathway[4].

Consensus of genes involved in DDR pathway - cancer

In Fig. 2b we present the altered genes in the DDR pathway between colon, adrenocortical, pancreatic and thyroid cancer:

XRCC4 and XRCC5: Involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and somatic recombination. The XRCC4 is bound to DNA and to DNA ligase IV (LIG4). The LIG4 - XRCC4 complex is responsible for the NHEJ ligation step, and XRCC4 enhances the joining activity of LIG4.

RAD52: Involved in double-stranded break repair. It plays a central role in genetic recombination and DNA repair by promoting the annealing of complementary single-stranded DNA and by stimulation of the RAD51.

RPA4: Is an essential factor for DNA double-strand break repair and the cell cycle checkpoint activation. The encoded protein localizes DNA repair foci and may be involved in the cellular DNA damage response.

RFC4: The gene is involved in replication factor C present in mismatch repair, nucleotide excision repair and cell cycle pathways.

H2AFX: Is a hystone sensor of DSBs and is usually highly expressed in cancer[5]. H2AFX is activated immediately after the barrier to monitor and detect DSBs[6].

Consensus of genes involved in Apoptosis pathway - adenoma

In Fig. 2c we present the altered genes in the Apoptosis pathway between colon, adrenocortical, pancreatic and thyroid adenoma:

CFLAR: This gene participates in the extrinsic pathways of apoptosis as a regulator of CASP8 which is responsible for activating cell death induced by TNFRSF1A[7,8]. Some evidences suggest that the CFLAR may facilitate the apoptosis forming a heterodimer with CASP8 which helps achieve the initial cleavage step of Procaspases 8[9].

CASP8: Most upstream protease of the activation cascade of caspases responsible for the TNFRSF6/FAS mediated and TNFRSF1A induced cell death. Binding to the adapter molecule FADD recruits it to either receptor. The resulting aggregate called death- inducing signaling complex (DISC) performs CASP8 proteolytic activation. The activation of CASP8 induces apoptosis and is regulated by PAK2[10].

FAS: Belongs to the tumor necrosis factor receptor family, this protein is part of the death-inducing signaling complex (DISC) that activates CASP8 to mediate apoptosis[11].

RIPK1: Is a regulator of the caspase cascade in TNFR1 and TNFR2 signaling subpathways to activate CASP8.

TNFRSF1B: Participates in the TNFR2 signaling subpathway and uses TANK as an apoptotic suppressor of BIRC3 that participates in the Caspase cascade in apoptosis and TNFR1 signaling subpathways.

TANK: Is a receptor of the TNFR2 that is induced by RIPK1 that controls the activation of MAP3K1[12].

PSMC2: Involved in the degradation of proteins, it participates in the regulation of apoptosis and cell cycle.

PAK2: Regulated by caspases and regulates apoptotic events [13].

DAPK2: Associated with cell death by two kinases and is a positive regulator of apoptosis[14].

BAX and BCL2: Participate of the apoptosis signaling in response to DNA damage subpathway and activate CASP3[15,16].

CDK6 and CDKN1A: Are cyclin kinases that work with p53 in cell cycle arrest in response to DNA damage[17]. CDK6 is inhibited by CDKN1A to induce cell cycle arrest in the G1/S checkpoint[18]. The inhibition of cell cycle in G2/M is directly induced by CDKN1A which is also regulated by the checkpoint kinase WEE1 responsible for controlling the transition from the G2/M checkpoint.

NEMO: Controls the mechanisms of response to a high level of DNA damages and is activated by the H2AFX gene and the ATM[19].

MDM2: Binds to the N-terminal end of P53 to inhibit its transcription.

BIRC3: Encodes CIPA2 and is also responsible for the inhibition of apoptosis through the activation of NF-kB and contributes to prevent activation of CASP8 through RIPK1[20].

MAP2K4: This kinase is a direct activator of MAP kinases in response to various environmental stresses or mitogenic stimuli.

MAP3K1: Component of a protein kinase signal transduction

cascade. It activates the ERK and JNK kinase pathways by phosphorylation of MAP2K1 and MAP2K4. It activates CHUK and NEMO, the central protein kinases of the activation NF-kB.

CHUK: Acts as part of the IKK complex in the conventional activation NF-kB pathway and phosphorylates inhibitors of NF-kB, thus leading to the dissociation of the inhibitor/NF-kB complex and ultimately, the degradation of the inhibitor.

CASP2: Involved in the activation cascade of caspases responsible for apoptosis execution. It might function by either activating some proteins required for cell death or inactivating proteins necessary for cell survival.

TNFSF10: Binds to TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D and possibly also to TNFRSF11B. It induces apoptosis. Its activity may be modulated by binding to the decoy receptors TNFRSF10C, TNFRSF10D and TNFRSF11B that cannot induce apoptosis.

XIAP: Apoptotic suppressor. It has E3 ubiquitin-protein ligase activity. It mediates the proteasomal degradation of target proteins, such as CASP3, SMAC or AIFM1. It is an inhibitor of CASP3, CASP7 and CASP9. It mediates activation of MAP3K7, leading to the activation of NF-kB.

ERC1: The protein encoded by this gene is a member of a family of RIM-binding proteins. RIMs are active zone proteins that regulate neurotransmitter release.

PIAS4: Also known as protein inhibitor of activated STAT, protein 4, or protein inhibitor of activated STAT, protein gamma is an enzyme that in humans is encoded by the PIAS4 gene.

Consensus of genes involved in Apoptosis pathway - cancer

In Fig. 2d we present the altered genes in the Apoptosis pathway between colon, adrenocortical, pancreatic and thyroid cancer:

CDK6: The protein encoded by this gene is a member of the cyclin-dependent kinase protein (CDK) family. This kinase is a catalytic subunit of the kinase protein complex that is important for the cell cycle G1 phase progression and G1/S transition. The activity of this kinase first appears in G1 phase, which is controlled by the regulatory subunits including D-type cycles and members of INK4 family of CDK inhibitors.

UNC5A: Acts as a dependence receptor required for apoptosis induction when not associated with netrin ligand.

RPA4: This gene encodes a single-stranded DNA-binding protein that is the 30-kDa subunit of the replication protein A complex. Replication protein A is an essential factor for DNA double-strand break repair and cell cycle checkpoint activation. The encoded protein localizes to DNA repair foci and may be involved in the cellular DNA damage response.

PAK2: Regulated by caspases and regulates apoptotic events[13].

ATR: Is a kinase protein which activates checkpoint signaling upon genotoxic stresses such as ionizing radiation (IR), ultraviolet light (UV), or DNA replication stalling, thereby acting as a DNA damage sensor. It actives the proteins: BRCA1, CHEK1, MDM2, RAD17, RPA2, SMC1 and TP53, which collectively inhibit DNA replication and mitosis and promote DNA repair, recombination and apoptosis. Phosphorylates by H2AFX at sites of DNA damage thereby, regulating DNA damage[1].

CCND1: Essential for the control of the cell cycle at the G1/S transition. It participates in the replicative senescence and cell cycle.

TP53: Acts as a tumor suppressor in many tumor types, it induces cell cycle arrest or apoptosis depending on the physiological circumstances and cell type. It is involved in cell cycle regulation as an activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases.

NEMO: Controls the mechanisms of response to a high level of DNA damages and is activated by the H2AFX gene and the ATM [19].

H2AFX: Is a hystone sensor of DSBs and is usually highly expressed in cancer[5]. H2AFX is activated immediately after the barrier to monitor and detect DSBs[6].

Consensus of genes involved in Cell cycle pathway - adenoma

In Fig. 2e we present the altered genes in the Apoptosis pathway between colon, adrenocortical, pancreatic and thyroid adenoma:

SMC3: Involved in structural maintenance of chromosomes and assembly of the mitotic spindle (Strunnikov et al. 1999).

EP300: Form a point of crosstalk between the P53 and NF-kB activation pathway[21].

TP53: Acts as a tumor suppressor in many tumor types; it induces growth arrest or apoptosis depending on the physiological circumstances and cell type. It is involved in cell cycle regulation as an activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases.

PSMC2: Involved in the degradation of proteins, it participates in the regulation of apoptosis and cell cycle.

CDC7: Altered in both, the Mitotic M-M/G1 phases and G2/M checkpoints subpathways. CDC7 is a kinase that activates proteins involved in cell cycle. In tumors this protein causes cell death by P53-independent apoptosis[22].

EED: Encodes multimeric proteins that are involved in the regulation of other genes and APC has regulatory functions and is also a tumor suppressor.

WEE1: Inhibits cyclin kinase in the G2 phase (Sorensen et al. 2012).

PAFAH1B1: This locus was identified as encoding a gene that when mutated or lost caused the lissencephaly associated with Miller-Dieker lissencephaly syndrome.

CDC2: Plays a key role in the control of the eukaryotic cell cycle. It is required in higher cells for entry into S-phase and mitosis. P34 is a component of the kinase complex that phosphorylates the repetitive C-terminus of RNA polymerase II

CLASP1: Required for the polarization of the cytoplasmic microtubule arrays in migrating cells towards the leading edge of the cell. It may act at the cell cortex to enhance the frequency of rescue of depolymerizing microtubules, by attaching their plus-ends to cortical platforms composed of ERC1 and PHLDB2.

MAD2L1: Execution of the mitotic checkpoint which monitors the process of kinetochore- spindle attachment and inhibits the activity of the anaphase, promoting complex by sequestering CDC20 until all chromosomes are aligned at the

metaphase plate.

Consensus of genes involved in Cell cycle pathway - cancer

In Fig. 2f we present the altered genes in the Apoptosis pathway between colon, adrenocortical, pancreatic and thyroid cancer:

CREBBP and EP300: Form a point of crosstalk between the P53 and NF-kB activation pathway[21].

EED: Encodes multimeric proteins that are involved in the regulation of other genes and APC has regulatory functions and is also a tumor suppressor.

RPA4: This gene encodes a single-stranded DNA-binding protein that is the 30-kDa subunit of the replication protein A complex. Replication protein A is an essential factor for DNA double-strand break repair and cell cycle checkpoint activation. The encoded protein localizes to DNA repair foci and may be involved in the cellular DNA damage response.

APC: Tumor suppressor. Promotes rapid degradation of CTNNB1 and participates in Wnt signaling as a negative regulator. APC activity is correlated with its phosphorylation state.

TP53: Acts as a tumor suppressor in many tumor types; it induces growth arrest or apoptosis depending on the physiological circumstances and cell type. It is involved in cell cycle regulation as an activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases.

RFCA: The gene is involved in replication factor C present in mismatch repair, nucleotide excision repair and cell cycle pathways.

Discussion of the fold change analysis in Colorectal inflammation

In the analysis of altered subpathways in colorectal inflammation, we observed alterations in three apoptosis subpathways: Apoptosis - Homo sapiens (KEGG), Granzyme A mediated apoptosis and replicative senescence (see Fig. 1). We can explain these alterations as a consequence of the dynamics of colorectal cell renewal, as it seems to be involved in inflammatory bowel diseases, considering that apoptosis and proliferation of epithelial cells can be found in colitis[23] and are involved in the regulation and activation of inflammatory processes characteristic of ulcerative colitis (UC) and Crohn's disease (CD) [23,24,25,26]. Though Apoptosis - homo sapiens (KEGG) and granzyme A subpathways are directly involved in apoptosis, the Replicative senescence subpathway also highlights the permanent cell cycle arrest as another barrier to prevent the progression of DNA damage in cells of this tissue. The importance of the senescent state in colorectal inflammation increases since telomere shortening is one of the factors that contribute to the progression of UC for colorectal carcinoma[27]. Furthermore, these dysplasias are the major causes of telomere shortening, DNA damage and senescence in ulcerative colitis[28]. In addition, from the analysis of differentially expressed genes in the replicative senescence pathway we find CDK6, CDC25A and CDKN1A altered, indicating the regulation of the senescent state in colorectal inflammation by CDKN1A. In the Apoptosis - homo sapiens (KEGG) subpathway TNFRSF10D/B, BIRC3, NTRK1 and BAX are differentially expressed. The genes TNFRSF10D/B encoding the receptors TRAILR2 and TRAILR4 - whose interaction between them is capable of inhibiting the apoptosis from the TRAIL ligand - regulate the inhibition of apoptosis through the activation of NF-kB[29]. The gene BIRC3 encodes CIPA2 and is also responsible for the inhibition of apoptosis through of activation NF-kB and contributes to prevent activation of CASP8 through RIPK1[20]. While these genes inhibit apoptosis, the pro-apoptotic gene BAX is maintained inactive by proteins of the BCL2 family. Yet, this inhibition decreases by the activation of BH3 (Bid, Bim and Puma) as well as by direct activation of BAX by BH3 members (Lindsay et al. 2011). Although in colorectal inflammation both apoptosis and proliferation can be active, the activation of TRAILR2 and BAX may also indicate the regulation of programmed cell death from the intrinsic pathway of apoptosis. NTRK1 (or TRKA) is an oncogene originally related with colorectal carcinoma but it is also a receptor for nerve growth factor (NGF)[30]. The expression of NGF and TRKA appear increased in patients suffering from UC, CD and inflammatory bowel, suggesting the activation of a pathway containing these two elements. The immune cells present in the intestinal wall express high levels of NGF and TRKA inducing the activation of the sensory nerve fibers which in turn, act to maintain the integrity of the intestinal mucosa[31,32]. The activity of Apoptosis Homo sapiens (KEGG) subpathway indicates the activation of extrinsic and intrinsic apoptosis through the expression of TNFRSF10D/B and BAX respectively, a third subpathway indicated by the activity of the granzyme A mediated apoptosis subpathway involves the activation of cytotoxic T cells whose main function is to regulate the onset of apoptosis in target cells by exposing these cells to toxic proteins including perforin. The main function of these proteins is to form pores in the plasma membrane in order to assist the penetration of granzymes released by T cells into the cytoplasm of cells marked for removal by apoptosis including tumor cells[33,34]. T lymphocytes express abundantly the granzymes A and B that coordinate apoptosis by two distinct pathways whereas the apoptotic activity of granzyme A is independent of the activation of caspases, the granzyme B pathway involves mitochondria and the rapid activation of caspases[35]. granzyme A regulates programmed cellular death by fragmentation of DNA, so the regulation of this process involves a complex released from the endoplasmic reticulum formed by s SET, APEX1, PP32, HMG2, NM23-H1 and TREX1, whose cleavage of HMG2, ROLE and SET by granzyme A, releases the fragmentation of DNA by NM23-H1 and TREX1[33,35]. The DEG analysis of the Granzyme A mediated apoptosis subpathway yielded four differentially expressed genes: SET, HMGB2, APEX1 and ANP32A indicating that in the colorectal inflammation the activation of apoptosis is also coordinated by T cells by the subpathway containing granzyme A, reinforcing the apoptotic activity of cells present in colorectal inflammation.

[a] *Departamento de Física, Universidade Federal de Santa Maria, Brazil. Tel: +55-55-3220-9521; E-mail: jcmombach@ufsm.br*
[b] *Departamento de Genética , Universidade Federal do Rio Grande do Sul, Brazi,.*
[c] *Universidade Federal do Pampa, Campus São Gabriel, Brazil.*

[d] *Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Brazil,.*
[e] *Departamento de Eletrônica e Computação, Universidade Federal de Santa Maria, Santa Maria, Brazil.*
[f] *Instituto de informática, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.*

1 T.H. Stracker and J.H.J. *Nature*, 2011, **12**, 90-103.
2 J.A. Duncan, J.R.Reeves and T.G. Cooke, *Clin Pathol: Mol Pathol*, 1998, **51**, 237–247.
3 S. Sengupta S and C.C. Harris, *Narute*, 2005, **6**, 44-55.
4 J. H. Lee, A.A. Goodarzi, P.A. Jeggo and T.T. Paull, *The Embo Journal*, 2010, **29**, 574-585.
5 W. Bonner, C. Redon, J. Dickey, A. Nakamura, O. Sedelnikova, S. Solier and Y. Pommier Y, *Nat Rev Cancer*, 2008, **8**, 957-967.
6 J.H. Kim, S.S. Kim, S.W. Byun, Y.J. Chang, J.S.Kim, J.K. Kim, H.J. Cho, K.W. Lim and E.S. Jung, *Journal of Gastroenterology*, 2010, **55**, 21-25.
7 W. Yeh W, L. Itie, A.J. Elia, M. Ng, H.B. Shu, A. Wakeham, C. Mirtsos, N. Suzuki, M. Bonnard, D.V. Goeddel and T.W. Mak, *Immunity*, 2000, **12**, 633-642.
8 K. Kawamura, N. Kawamura, J. Kumagai, J. Fukuda and T.Tanaka, *Biology of Reproduction*, 2007, **76**, 611-618.
9 G. Jing, K. Yuan, Q. Liang, Y. Sun, X. Mao, J.M. Mcdonald and Y. Chean, *Laboratory Investigation*, 2012, **92**, 82–90.
10 Y. Hsuuw, T. Kuo, K. Lee, Y. Liu, Y. Huang and C. Lai, *Annals of the New York Academy of Sciences*, 2009, **1171**, 501–508.
11 O. Micheau, M. Thome, P. Schneider, N. Holler, J. Tschopp, D.W. Nicholson, C. Briand and M.G.Grutter, *The Journal of Biological Chemistry*, 2002, **277**, 45162-45171.
12 K.M. Kaye, O. Devergnei, J.N. Harada, K.M. Izumit, R. Yalamanchilit, E. Kiefft and L. Mosialost, *Medical Sciences*, 1996, **93**, 11085-11090.
13 E. Manser, C. Chong, Z.S. Zhao, T. Leung, G. Michae, C. Hall and L. Lim, *The Journal of Biological Chemistry*, 1995, **270**, 5070–5078.
14 T. Kawai, F. Nomura, K. Hoshino, N.G. Copeland, D.G. Gilbert, N.A. Jenkins and S. Akira , *Oncogene*, 1998, **18,** 3471-3480.
15 L. Scorrano, S.A. Oakes, J.T. Opferman, E.H. Cheng, M.D. Sorcinelli, T. Pozzan and S.J. Korsmeyer, *Science*, 2003, **300**, 135-139.
16 S.J. Hong, R.J. Sung, H. In Yang, C. Shik Yin, H.G. Koh, M. Jang, C. Kim, B. Choe and J. Chung J, *Toxicon*, 2005, **46**, 39–45.
17 H.C. Reinhardt and B. Schumacher, *Trends in Genetics*, 2012, **28**, 128-136.
18 G. P. Studzinski, X. Wang and M. Danilenko, *Cancer Biol Ther*, 2010, **9**, 253-255
19 S. Biton and A. Ashkenazi, *Cell*, 2011, **145**, 92-103.
20 M. Gyrd-Hansen and P. Meier, *Nature Reviews Cancer*, 2010, **10**, 561 – 574.
21 A. Dey, V. Tergaonkar V and D.P. Lane, *Nature*, 2008, **7**, 1031-1040.
22 K. Labib, *Cancer Research*, 2010, **24**, 1208–1219.
23 L. Chen, S.M. Park, J.R. Turner and M.E. Peter, *Inflamm. Bowel Dis.* **16**, 1071 - 1076.
24 S. Danese and C. Fiocchi, *N. Engl. J. Med.*, 2011, **365**, 1713-25.
25 A. Di Sabatino, P. Biancheri, L. Rovedatti, T.T. Macdonald and G.R. Corazza, *Intern. Emerg. Med*, 2011, **7**, 103-111.
26 S. Kraus and N. Arber, *Current Opinion in Pharmacology*, 2009, **9**, 405–410.
27 G. Aubert and P.M. Lansdorp, *Physiol. Rev*, 2008, **88**, 557–579.
29 C. Falschlehmer, C.H. Emmerich, B. Gerlach and H. Walczak, *The International Journal of Biochemistry & Cell Biology*, 2007, **39**, 1462–1475.
30 L. Alberti, C. Carniti, C. Miranda, E. Roccato and M.A. Pierotti, *Journal of Cellular Physiology*, 2003, **195**, 168–186.
31 F.F.Di Mola, F. Friess, W.Z. Zhu, A. Koliopanos, T. Bley, P. Di Sebastiano, P. Innocenti, A. Zimmermann and M.W. Büchler, *Gut*, 2000, **46**, 670–678.
32 L. Qiao L and J.R. Grider, *Pain*, 2010, **151** 117–127.
33 S. Elmore, *Toxicol. Pathol*, **35**, 495 – 516.
34 Z. Fan and Q. Zhang, *Cellular & Molecular Immunology*, 2005, **2**, 259 – 264.
35 M. Bots and J. Medema, *Journal of Cell Science*, 2006, **119**, 5011-5014.