The role of DNA methylation in the development of colorectal cancer

Ph.D. thesis

Árpád Patai, M.D.

Semmelweis University
Clinical Medicine Doctoral School

Supervisor: Béla Molnár, M.D., D.Sc.

Official reviewers:
Zoltán Rakonczay, M.D., D.Sc.
Csaba Bödör, M.D., Ph.D.

Head of the Complex Examination:
András Arató, M.D., D.Sc.
Members of the Complex Examination Committee:
Kálmán Tory, M.D., Ph.D.
László Szabó, M.D., Ph.D.
Tivadar Tulassay, M.D., D.Sc.

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INTRODUCTION

Colorectal cancer (CRC) is a heterogeneous disease and consists of distinct molecular and clinicopathological subtypes.

80-85% of CRCs develop via the adenoma-carcinoma sequence (ACS), where subsequent genetic mutations (inactivating mutations of tumor suppressors, e.g. *APC*, *SMAD4* and *TP53,* and activating mutations of oncogenes, e.g. *KRAS, PIK3CA*) drive from healthy colonic mucosa through preneoplastic adenomas with low-grade and high-grade dysplasia to invasive CRC.

Epigenetic alterations (e.g. DNA methylation) are an order of magnitude more common in CRC, than genetic mutations. The aberrant hypermethylation in the promoter region of certain genes – via a different mechanism as observed in mutations – cause inactivation of those genes. 20-30% of CRCs have a high frequency of methylated genes, hence this subtype has been termed as CpG island methylator phenotype (CIMP), which is more common in elderly women.

The third subtype termed microsatellite instable (MSI) consists 10-20% of CRCs, and is characterized by the functional loss of mismatch repair (MMR) enzymes caused by hypermethylation of the respective genes (e.g. *MLH1*), so a part of sporadic MSI cancers are also a part of CIMP subgroup. Wnt pathway has a pivotal role in the development of CRC, its aberrant activation (e.g. via

mutation of *APC* or via the hypermethylation of *SFRP* (secreted frizzled-related protein)) leads to colorectal cancer.

Precancerous lesions arising via the adenoma-carcinoma sequence are called adenomatous polyps, whereas forerunners of the CIMP pathway are termed serrated polyps (traditional serrated adenoma, TSA; and sessile serrated adenoma, SSA).

Chronic exposure to several carcinogenic agents (e.g. chronic inflammation, smoking) through a genetically and epigenetically altered field can predispose the macroscopically and histologically normal appearing mucosa to the development of multiple primary tumors. This phenomenon is termed as field effect or field cancerization.

OBJECTIVES

In my work, DNA methylation alterations in different stages of colorectal carciogenesis have been studied, along with its effect on gene and protein expression.

Our aims were as follow:

- to analyze DNA methylation alterations of 96 genes, and to study 12 common mutations in
  - healthy colonic samples,
  - precancerous lesions (traditional and serrated adenomas),
  - inflammatory bowel disease,
  - CRC,
  - normal adjacent tissue to CRC

- to analyze the effect of DNA methylation on gene and protein expression
- to examine DNA methylation alterations in WNT pathway in the microenvironment of CRC
- to study gene expression changes as response to demethylation treatment CRC cell line

METHODS

Patients

For the DNA methylation analysis clinical samples were collected during routine colonscopy. Altogether 78 samples were analyzed, including adults (older than 18 years, N, n=5) and young patients (younger than 18 years, Y, n=5), tubular adenoma with low-grade dysplasia (LGD, n=17), adenoma with high-grade dysplasia (HGD, n=6), CRC (n=17), metastatic CRC (MCRC, n=7), active UC (UCa, n=4), inactive and long-standing UC (UCi, n=4). Field effect was studied by taking samples from at least 10 cm (cancer normal, CN) and 1 cm away (field, F) from the macroscopically visible margin of the tumor.

DNA methylation array analysis

DNA was extracted using High Pure PCR Template Preparation Kit, and DNA methylation profiles were examined using the EpiTect Methyl qPCR Array System.

RNA extraction and whole genomic mRNA expression microarray analysis

Total RNA was extracted by using RNeasy Mini Kit, and whole genomic mRNA expression microarray analysis was performed using HGU133 Plus2.0 microarray.

Immunohistochemistry analysis of MMR proteins and SFRP1

Tissue microarrays (TMAs) were constructed with 2 mm cores from formalin-fixed and paraffin-embedded tissues. TMA sections were fixed on slides, and immunohistochemistry analysis for MLH1, MSH2, MSH6, PMS2, SFRP1 protein expression was performed. Following digital archiving, stainings were evaluated with a Pannoramic Viewer digital microscope.

Analysis of SFRP1 (secreted frizzled-related protein 1) expression in myofibroblasts

To analyze stromal myofibroblasts, an α-SMA-SFRP1 protein dual fluorescent staining was used in normal biopsy samples (n=20), surgically removed CRC (n=35) and colonic tissues containing NAT and CRC areas (n=14).

Demethylation treatment in cell lines

HT29 colon adenocarcinoma cell line was treated with a demethylation agent, 5-aza-2'-deoxycytidine, then RNA was extracted and whole genome mRNA expression

analysis was performed. The effect of 5-aza-2'-deoxycytidine/trichostatin A treatment on whole genome mRNA expression was also examined *in silico* on a molecularly distinct CRC cell line, HCT116.

Analysis of genetic mutations by next generation sequencing

Mutation hotspots of 12 frequently described gene mutations in CRC (*APC, BRAF, CTNNB1, EGFR, FBXW7, KRAS, MSH6, NRAS, PIK3CA, SMAD2, SMAD4, TP53*) were amplified by own designed PCR primers. Amplicons were sequenced with a GS Junior Instrument, GS Junior Titanium Sequencing Kit was used for bead enrichment and sequencing steps. Variants were identified with the Amplicon Variant Analyzer software.

RESULTS

Comparison of methylation profiles between subgroups

Comparison of methylation profiles between CRCs, precancerous lesions, inflamed and normal colorectal mucosa revealed that CRC and precancerous lesions have a characteristic methylation signature. We identified a set of 10 genes that were hypermethylated in at least 85% of tumor samples. These genes were not hypermethylated in MCRC, indicating that metastatic CRCs have a different epigenetic signature.

DNA methylation profiling revealed an increasing number of methylated genes along ACS. In addition to the 9 hypermethylated genes found in the majority (>95%) of samples, including normal and UC tissue, additional 34, 46 and 32 hypermethylated genes were observed in more than half of LGD, HGD and CRC samples, respectively. In MCRC only 2 additional hypermethylated genes were observed. A set of 12 genes were exclusively hypermethylated in HGD. The frequency of hypermethylated DNA copies was also significantly higher in the premalignant alterations than in cancer.

We also analyzed the methylation profile of 4 samples with UCa and 4 samples with UCi. Although we found difference between methylation levels (e.g. 63% vs 80%), both conditions showed a similar pattern to that of normal samples, no significant changes were observed and no additional gene was identified as hypermethylated.

Validation of DNA methylation results

The level of DNA methylation was higher in precancerous lesions (LGD and HGD), compared to CRC. This observation was further validated in 7 synchronous LGD-CRC pairs where methylation levels proved to be greater in LGD than in synchronous CRC. This observation was also confirmed by bisulfite-HRM analysis in 3 genes and showed consistent results. The

number of hypermethylated genes was also higher in LGD, than in synchronous CRC. The mean DNA methylation percentage of methylated genes was significantly higher in LGD than in CRC. The comparison of DNA methylation profiles between CRC and NAT revealed that hypermethylation occurred only in cancer but not in the adjacent field.

Correlation between DNA methylation, mRNA and protein expression

To analyze the effect of DNA hypermethylation, we examined mRNA and protein expression levels of SFRP1, a well-described antagonist of the Wnt pathway, frequently aberrantly activated in CRC. We confirmed the findings of previous studies, that DNA hypermethylation leads to subsequent underexpression of *SFRP1* mRNA and lower protein levels in CRC, and also in precursor lesions (LGD, HGD, serrated polyps).

Mutation analysis in adenoma-carcinoma sequence and sessile serrated adenoma

The most frequent mutation (*APC*, *TP53*, *KRAS*, *SMAD4*, *BRAF*) occured more common in CRC (6-53%), than in traditional adenomas (LGD and HGD, 4-30%). *BRAF* mutation was most prevalent (18%) in serrated adenomas.

DNA methylation analysis of serrated polyps

When compared to normal samples, besides the 9 universally hypermethylated genes, there were 12 genes (*CALCA, DKK2, GALR2, OPCML, PCDH10, SFRP1, SFRP2, SLIT3, SST, TAC1, VIM, WIF1*) to be methylated in all all SSAs, and 2 additional genes (*BNC1* and *PDLIM4*) were hypermethylated in 3 out of 4 SSAs, but in none of the normal samples. The sole TSA sample showed hypermethylation for only two genes (*CALCA* and *SST*), which the only two genes that were hypermethylated in all serrated polyps.

Reduced stromal SFRP1 mRNA expression in CRC compared to NAT

Macrodissected NAT samples showed significantly ($p<0,001$) higher *SFRP1* mRNA expression than CRC samples (Figure 1/A).

Using laser microdissected tissue compartments, a significantly ($p<0.001$) higher *SFRP1* mRNA expression was found in the NAT epithelium compared to CRC epithelium: the average gene expression values in CRC was one-third of the average expression values observed in NAT samples.

SFRP1 protein expression of myofibroblasts in normal, NAT and CRC

Strong stromal SFRP1 protein expression was found in normal, NAT and transitional area between NAT and

CRC, which localized mainly in the pericryptal region. These stromal SFRP1-positive cells were identified as α-SMA-positive myofibroblasts in NAT by dual fluorescent immunohistochemistry. In NAT regions pericryptal α-SMA-positive cells showed strong SFRP1 protein expression (85.73%±12.61%/all α-SMA-positive and SFRP1 double positive cells/ α-SMA-positive cells in stroma). In most of the analyzed CRC samples heterogeneous SFRP1 expression was detected, the number of α-SMA-positive cells increased significantly, but the SFRP1 protein expression was significantly reduced (27.65%±18.27%) in representative tumoral areas as compared to NAT ($p<0.001$). Decreased SFRP1 expression was observed in NAT samples. Parallel with the disappearance of epithelial SFRP1 protein expression, subepithelial myofibroblasts showed retained protein expression in these areas. NAT regions which localized more distantly from CRC showed similar SFRP1 protein expression patterns than normal samples. Decreased epithelial SFRP1 protein expression was detected in 71.42% of CRC samples.

SFRP1 promoter hypermethylation in the NAT area and CRC

The methylation status of α-SMA immunopositive stromal myofibroblasts was examined in laser microdissected cells from NAT and CRC samples.

SFRP1 was partially (heterogeneously) hypermethylated in stromal myofibroblasts from CRC, whereas unmethylated myofibroblasts from NAT. Decreased epithelial SFRP1 protein expression was found in crypts of most NAT samples which localized closely to the tumor. This decreased protein expression was related to epigenetic silencing of *SFRP1*.

The effect of demethylation treatment on mRNA expression level of hypermethylated genes

Whole genomic mRNA expression microarray data was utilized to analyze mRNA expression of 7 genes (*MAL*, *PCDH10*, *PDLIM4*, *SFRP1*, *SLIT2*, *SST*, *TMEFF2*) previously identified as hypermethylated in CRC. These genes showed decreased mRNA expression in CRC compared to normal tissue in the majority of the examined samples.

To examine whether downregulation of these gene can be reversed by demethylation, 5-aza-2'-deoxycytidine treatment was applied to HT-29 cells, which partly restored mRNA expression of these genes.

Similar tendency was oberved in another *in silico* analysis, where 5-aza-2'-deoxycytidine/trichostatin A treatment was applied to HCT116 cell line.

CONCLUSIONS

Methylation status of 96 genes were analyzed. Our results indicate that there is a characteristic methylation

pattern for CRC and also for their precursor lesions. We identified a set of 10 genes (*SFRP1*, *SST*, *BNC1*, *MAL*, *SLIT2*, *SFRP2*, *SLIT3*, *ALDH1A3*, *TMEFF2*, *WIF1*) that are frequently hypermethylated in benign and malignant colorectal tumors. More interestingly precursor lesions had more hypermethylated genes and a higher grade of methylation than CRC, when synchronous LGD and CRC pairs were compared. We confirmed that metastatic CRCs have a different methylation fingerprint, as most of the genes hypermethylated in precancerous and cancerous lesions were not methylated in MCRC.

We showed that 7 hypermethylated genes had decreased mRNA expression in tumorous samples, and this decreased mRNA expression could be partly restored by demethylation treatment.

Among these, SFRP1 was analyzed also at the protein level and showed decreased protein expression compared to healthy samples.

When the frequency of mutations and hypermethylated genes in tumorous samples was compared, methylated genes showed a significantly higher penetrance than mutations. We identified 10 genes that were hypermethylated in more than 85% of the examined samples, whereas the frequency of the most common mutations was less (0-50%).

6 genes (*BNC1*, *SFRP1*, *SFRP2*, *SLIT3*, *SST*, *WIF1*) were found to be frequently hypermethylated not only in CRC, but in both traditional and serrated pre-neoplastic

lesions, that warrants further investigation with larger sample size.

BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

Publications related to PhD thesis

1. **Patai ÁV*** and Barták BK* and Péterfia B*, Micsik T, Horváth R, Sumánszki C, Péter Z, Patai Á, Valcz G, Kalmár A, Tóth K, Krenács T, Tulassay Z, Molnár B. (2017) Comprehensive DNA methylation and mutation analyses reveal a methylation signature in colorectal sessile serrated adenomas. Pathol Oncol Res, 23: 589-594. *co-first authors (IF: 1.736)
2. **Patai ÁV**, Valcz G, Hollósi P, Kalmár A, Péterfia B, Patai Á, Wichmann B, Spisák S, Barták BK, Leiszter K, Tóth K, Sipos F, Kovalszky I, Péter Z, Miheller P, Tulassay Z, Molnár B. (2015) Comprehensive DNA methylation analysis reveals a common ten-gene methylation signature in colorectal adenomas and carcinomas. PLoS One, 10: e0133836. (IF: 3.057)
3. Valcz G* and **Patai ÁV***, Kalmár A, Péterfia B, Fűri I, Wichmann B, Műzes G, Sipos F, Krenács T, Mihály E, Spisák S, Molnár B, Tulassay Z. (2014) Myofibroblast-derived SFRP1 as potential inhibitor of colorectal carcinoma field effect. PLoS One, 9: e106143. *co-first authors (IF: 3.234)
4. **Patai ÁV*** and Sumánszki C*, Sipos F, Molnár B, Tulassay Z. A DNS-metiláció szerepe és klinikai

jelentősége a vastagbélrák kialakulásában. (2015) Magy Belorv Arch, 68: 163-168. *co-first authors (IF: 0)

5. **Patai ÁV**, Micsik T, Péter Z, Horváth R, Molnár B, Tulassay Z. (2014) A fogazott polypok jelentősége a vastagbélrák kialakulásában. Magy Belorv Arch, 67: 225-233. (IF: 0)
6. **Patai AV**, Molnár B, Tulassay Z, Sipos F. Serrated pathway: alternative route to colorectal cancer. (reprint in book chapter) (2014) World Clinical Gastroenterology, 1: 241-249. (IF: 0)
7. **Patai AV**, Molnár B, Tulassay Z, Sipos F. (2013) Serrated pathway: alternative route to colorectal cancer. World J Gastroenterol, 19: 607-615. (IF: 2.433)
8. **Patai AV*** and Molnár B*, Kalmár A, Schöller A, Tóth K, Tulassay Z. (2012) Role of DNA methylation in colorectal carcinogenesis. Dig Dis. 2012; 30: 310-315. *co-first authors (IF: 2.725)
9. Barták BK, Kalmár A, Péterfia B, **Patai ÁV**, Galamb O, Valcz G, Spisák S, Wichmann B, Nagy ZB, Tóth K, Tulassay Z, Igaz P, Molnár B. (2017) Colorectal adenoma and cancer detection based on altered methylation pattern of SFRP1, SFRP2, SDC2, and PRIMA1 in plasma samples. Epigenetics, 12: 751-763. (IF: 4.394)
10. Tóth K, Patai ÁV, Kalmár A, Barták BK, Nagy ZB, Galamb O, Wichmann B, Tulassay Z, Molnár B. (2017) Circadian Rhythm of Methylated Septin 9, Cell-Free

DNA Amount and Tumor Markers in Colorectal Cancer Patients. Pathol Oncol Res, 23: 699-706. (IF: 1.736)

11. Péterfia B, Kalmár A, **Patai ÁV**, Bodor A, Micsik T, Wichmann B, Egedi K, Hollósi P, Kovalszky I, Tulassay Z, Molnár B. (2017) Construction of a multiplex mutation hot spot PCR panel: the first step towards colorectal cancer genotyping on the GS Junior platform. J Cancer, 8: 162-173. (IF: 2.916)

12. Galamb O, Kalmár A, Barták BK, **Patai ÁV**, Leiszter K, Péterfia B, Wichmann B, Valcz G, Veres G, Tulassay Z, Molnár B. (2016) Aging related methylation influences the gene expression of key control genes in colorectal cancer and adenoma linked key control genes. World J Gastroenterol, 22: 10325-10340. (IF: 3.365)

13. Galamb O, Kalmár A, Péterfia B, Csabai I, Bodor A, Ribli D, Krenács T, **Patai ÁV**, Wichmann B, Barták BK, Tóth K, Valcz G, Spisák S, Tulassay Z, Molnár B. (2016) Aberrant DNA methylation of WNT pathway genes in the development and progression of CIMP-negative colorectal cancer. Epigenetics, 11: 588-602. (IF: 4.394)

14. Kalmár A, Péterfia B, Hollósi P, Galamb O, Spisák S, Wichmann B, Bodor A, Tóth K, **Patai ÁV**, Valcz G, Nagy ZB, Kubák V, Tulassay Z, Kovalszky I, Molnár B. (2015) DNA hypermethylation and decreased mRNA expression of MAL, PRIMA1, PTGDR and SFRP1 in colorectal adenoma and cancer. BMC Cancer, 15: 736. (IF: 3.265)

15. Kalmár A, Péterfia B, Wichmann B, **Patai ÁV**, Barták BK, Nagy ZB, Furi I, Tulassay Z, Molnár B. Comparison of automated and manual DNA isolation methods for DNA methylation analysis of biopsy, fresh frozen, and formalin-fixed, paraffin-embedded colorectal cancer samples. J Lab Autom, 20: 642-651. (IF: 1.297)

16. Kalmár A, Péterfia B, Hollósi P, Wichmann B, Bodor A, **Patai ÁV**, Schöller A, Krenács T, Tulassay Z, Molnár B. (2015) Bisulfite-based DNA methylation analysis from recent and archived formalin-fixed, paraffin embedded colorectal tissue samples. Pathol Oncol Res, 21: 1149-1156. (IF: 1.940)

17. Leiszter K, Sipos F, Galamb O, Krenács T, Veres G, Wichmann B, Fűri I, Kalmár A, **Patai ÁV**, Tóth K, Valcz G, Molnár B, Tulassay Z. (2015) Promoter hypermethylation-related reduced somatostatin production promotes uncontrolled cell proliferation in colorectal cancer. PLoS One, 10: e0118332. (IF: 3.057)

18. Tóth K, Wasserkort R, Sipos F, Kalmár A, Wichmann B, Leiszter K, Valcz G, Juhász M, Miheller P, **Patai ÁV**, Tulassay Z, Molnár B. (2014) Detection of methylated Septin 9 in tissue and plasma of colorectal patients with neoplasia and the relationship to the amount of circulating cell-free DNA. PLoS One, 9: e115415. (IF: 3.234)

19. Sipos F, Műzes G, **Patai AV**, Fűri I, Péterfia B, Hollósi P, Molnár B, Tulassay Z. (2013) Genome-wide screening

for understanding the role of DNA methylation in colorectal cancer. Epigenomics, 5: 569-581. (IF: 5.215)

20. Tóth K, Sipos F, Kalmár A, **Patai AV**, Wichmann B, Stoehr R, Golcher H, Schellerer V, Tulassay Z, Molnár B. (2012) Detection of methylated SEPT9 in plasma is a reliable screening method for both left- and right-sided colon cancers. PLoS One, 7: e46000. (IF: 3.730)

Other publications

21. Patai Á, Solymosi N, Mohácsi L, **Patai ÁV**. (2017) Indomethacin and diclofenac in the prevention of post-ERCP pancreatitis: a systematic review and meta-analysis of prospective controlled trials. Gastrointest Endosc, 85: 1144-1156.e1. (IF: 6.501)

22. Patai Á, **Patai ÁV**, Tulassay Z. (2017) Van-e kapcsolat az appendix és a colitis ulcerosa kóreredete között? Magy Belorv Arch. 2017; 70: 154-156. (IF: 0)

23. Patai Á, **Patai ÁV**. (2017) A gyomor-, bélrendszer rákbetegségeinek megelőzési stratégiái. A vastag- és végbélbélrák megelőzhető! (book chapter) Vas prevenció egészségfejlesztési füzetek I., 53-62. (IF: 0)

24. Németh BC, **Patai ÁV**, Sahin-Tóth M, Hegyi P. (2016) Misfolding cationic trypsinogen variant p.L104P causes hereditary pancreatitis. Gut, 66: 1727-1728. (IF: 16.658)

25. **Patai ÁV**, Patai Á. (2016) Rectal indomethacin for the prevention of post-ERCP pancreatitis. Gastroenterology, 151: 565-566. (IF: 18,392)

26. Valcz G, Galamb O, Krenács T, Spisák S, Kalmár A, **Patai ÁV**, Wichmann B, Dede K, Tulassay Z, Molnár B. (2016) Exosomes in colorectal carcinoma formation: ALIX under the magnifying glass. Mod Pathol, 29: 928-938. (IF: 5,728)

27. **Patai ÁV**, Molnár Cs, Csóka C, Micsik T, Hajdu-Andersson I, Farkas P, Herszényi L, Tulassay Z. (2016) Gyomorrák genetikája és molekuláris altípusainak jelentősége. Magy Belorv Arch, 69: 5-8. (IF: 0)

28. Csontos ÁA, **Patai ÁV**, Miheller P. (2015) Antiszensz oligonukleotid kezelés gyulladásos bélbetegségekben szenvedők körében. Magy Belorv Arch, 68: 351-353. (IF: 0)

29. **Patai ÁV*** and Péter Z*, Csontos ÁA, Tulassay Z. Ritka epeúti intervenciós megoldások endoszkópos retrográd kolangio-pankreatográfia során. Magy Belorv Arch, 68: 347-350. *co-first authors (IF: 0)

30. **Patai ÁV**, Csóka C, Micsik T, Miheller P, Csontos ÁA, Müllner K, Herszényi L, Tulassay Z. (2015) Melanoma malignum a vastagbélben. Magy Belorv Arch, 68: 303-306. (IF: 0)

31. Patai Á, **Patai ÁV**. (2015) Effect of acetylsalicylic acid, clopidogrel and indomethacin on bleeding after sphincterotomy: the good, the better and no-one is ugly. Endoscopy, 47: 1191 (IF: 5.634)

32. Patai Á, **Patai ÁV**, Solymosi N, Tulassay Z, Herszényi L. (2015) Az endoszkópos retrográd cholangiopancreatographiát követő heveny

hasnyálmirigy-gyulladás megelőzése. Orv Hetil, 156: 711-715. (IF: 0.291)

33. Takács T, Czakó L, Dubravcsik Z, Farkas G, Hegyi P, Hritz I, Kelemen D, Lásztity N, Morvay Z, Oláh A, Pap Á, Párniczky A, **Patai Á**, Sahin-Tóth M, Szentkereszti Z, Szmola R, Tiszlavicz L, Szücs Á; Magyar Hasnyálmirigy Munkacsoport. (2015) Krónikus pancreatitis. A Magyar Hasnyálmirigy Munkacsoport bizonyítékon alapuló kezelési irányelvei. Orv Hetil, 156: 262-288. (IF: 0.291)

34. Patai Á, Solymosi N, **Patai ÁV**. (2015) Effect of rectal indomethacin for preventing post-ERCP pancreatitis depends on difficulties of cannulation: results from a randomized study with sequential biliary intubation. J Clin Gastro, 49: 429-437. (IF: 3,163)

35. Patai Á, Solymosi N, **Patai ÁV**. (2014) Does rectal indomethacin given for prevention of post-ERCP pancreatitis increase bleeding after biliary endoscopic sphincterotomy or cardiovascular mortality. Post hoc analysis using prospective clinical data. Medicine, 93: e159. (IF: 5.723)

36. Leiszter K, Galamb O, Sipos F, Krenács T, Veres G, Wichmann B, Kalmár A, **Patai AV**, Tóth K, Valcz G, Molnár B, Tulassay Z. (2013) Sporadic colorectal cancer development shows rejuvenescence regarding epithelial proliferation and apoptosis. PLoS One, 8: e74140. (IF: 3.534)

37. Valcz G* and **Patai AV***, Kalmár A, Sipos F, Molnár B, Tulassay Z. (2012) A horizontális génátvitel szerepe a

tumorképződésben. Magy Belorv Arch, 65: 256-260. *co-first authors (IF: 0)

38. Fűri I, Sipos F, Spisák S, Kalmár A, **Patai ÁV**, Molnár B, Tulassay Z. (2012) A Toll-like receptor rendszer aktiválása nukleinsav-analógokkal gyulladásos és daganatos betegségek terápiájában. Magy Belorv Arch, 65: 249-255. (IF: 0)

39. Valcz G, Sipos F, Krenács T, Molnár J, **Patai AV**, Leiszter K, Tóth K, Wichmann B, Molnár B, Tulassay Z. (2012) Increase of α-SMA(+) and CK(+) cells as an early sign of epithelial-mesenchymal transition during colorectal carcinogenesis. Pathol Oncol Res, 18: 371-376. (IF: 1,555)

40. Valcz G, Krenács T, Sipos F, **Patai AV**, Wichmann B, Leiszter K, Tóth K, Balogh Z, Csizmadia A, Hagymási K, Masszi T, Molnár B, Tulassay Z. (2011) Lymphoid aggregates may contribute to the migration and epithelial commitment of bone marrow derived cells in colonic mucosa. J Clin Pathol, 64: 771-775. (IF: 2.306)

41. Valcz G, Sipos F, Krenács T, Molnár J, **Patai AV**, Leiszter K, Tóth K, Solymosi N, Galamb O, Molnár B, Tulassay Z. (2010) Elevated osteopontin expression and proliferative/apoptotic ratio in the colorectal adenoma-dysplasia-carcinoma sequence. Pathol Oncol Res, 16: 541-545. (IF: 1.483)

42. Leiszter K, Galamb O, Sipos F, Tóth K, Valcz G, **Patai VA**, Molnár J, Molnár B, Tulassay Z. (2010) Az öregedés mikroszkópos és molekuláris jelei a

vastagbélben, valamint ezek lehetséges szerepe az időskori vastagbélrák kialakulásában. Orv Hetil, 151: 885-892. (IF: 0)