K112371: Genomic landscape of human melanoma progression

Molecular epidemiology of melanoma in Hungary

Molecular epidemiology of cutaneous melanoma in Hungary
The major oncogenic drivers of cutaneous melanoma are mutant BRAF, NRAS and KIT (*Timar et al.Cancer Metastasis Rev2016*) While the data on BRAF mutation rate is quite homogenous worldwide (~50%) followed by NRAS (~20%), less data are available for KIT. In the rare ALM and mucosal melanomas KIT mutation is the predominant but the data are very heterogenous worldwide (10-50%). Meanwhile there are no valid data on the molecular epidemiology of cutaneous melanoma in Hungary. We have analysed our data on molecular testing of melanoma and found that based on evaluation of apr. **227** cases the BRAF mutation frequency (exon 15c600) is 46%, the NRAS mutation frequency is 21.3% correlating well with international data. Finally we have for the first time tested KIT mutations in a BRAF/NRAS double wt 79pts cohort of skin melanomas and 17 cases of mucosal melanomas. We have found that KIT mutation frequency is 43%, unexpectedly high, with higher frequency in non-UV acrolentiginous forms (58.8%) as compared to the UV-induced ones (31.1%). Calculating the KIT mutation frequency in skin melanoma it was detected a 14.9%, comparable to NRAS. In the double wildtype (BRAF/NRAS) mucosal cohort the KIT mutation rate was comparable (41.2%). In skin melanoma exon11 (44.7%) followed by exon9 (21,1%) were the most frequently involved ones in KIT. On the contrary, in mucosal melanoma exon 9 was most frequent involved followed by exon13/17. We have detected KIT mutation hotspots in exon9(c482/491/492), in exon11 (c559,570/572), in exon13 (c642), in exon17 (c822) and in exon18 (c853). It is of note that on the contrary to what the literature suggest, in Hungary the majority of KIT-mutations are classical activating mutations closely resembling GIST tumors, where KIT-inhibitors are the basis of therapy. Today the diagnostic routine is that only BRAF mutation is tested in case of cutaneous melanoma patients but the relatively high frequency of other targetable driver mutations in both the classical UV-induced and in the rare non-UV forms suggest to test all the tree oncogenes (BRAF, NRAS, KIT) routinely. (*Doma V et al. Pathol Oncol Res 2019*)

Metastatic melanoma biobank

We have completed now a 53 primary skin melanoma biobank where 148 corresponding various visceral metastases can be found. The bank is mostly paraffin embedded tissue samples but a significant number of cases are fresh frozen tissues collected at autopsy. After macrodissection of the primary tumors, collected samples underwent DNA isolation and quality control. Sanger sequencing was used to determine BRAF exon 15 mutations. Then, BRAF wild type cases were subjected to NRAS exon 2 and 3 sequencing, whereas double wild type cases C-KIT exon 11 and 13 direct sequencing. Overall, in our cohort the following mutations were observed: BRAF V600E, V600K, K601E, NRAS G12C, Q61K, Q61L and Q61R and no C-KIT mutation was detected. BRAF mutant tumors were the most frequent (~50%) followed by NRAS mutants (~20%).

Alterations in mutant allele frequency of oncogenic drivers BRAF/NRAS during melanoma progression

in both the primary- and metastatic tumors mutant variant allele frequencies (MAF) were re-calculated compensating for Tumor/Normal cell ratio, which was never done in previous reports. We have found that MAF increased significantly in metastatic tissues compared to primary tumors in case of BRAF mutant tumors exclusively which was due to significant increase in lung- and other than major site metastases (but not in CNS or liver metastases). Based on MAF values mutant alleles were classified as dominant (>50%), minor (15-50%) or subclonal (<15%). Decrease in MAF is a rare event during visceral metastatic progression of human melanoma and is independent from oncogenic drivers:

4/32 (12.5%) in BRAF-mutant and 2/12 (16.7%) in NRAS mutant melanoma. Stability of the MAF value is the characteristic of NRAS-mutant melanoma (6/12,50%) unlike of the BRAF-mutant tumors (10/32,31.3%) where increase in MAF is the most frequent genetic event (18/32,56.3%). It is another clinically relevant observation that in a significant proportion of melanoma (25%), the primary tumor contains only a subclonal driver mutation. Furthermore, MAF can change unpredictably in metastases and dominant to subclonal conversion is relatively frequent (>20%). Based on these observations we suggest to characterize driver status of the metastatic tissue rather than the primary before clinical decision making. Furthermore, it is clinically relevant to provide MAF values in the molecular pathology report in case of oncogenic driver mutations. *(Doma V et al. BMC Cancer 2019).*

We have analysed the genetic factors behind the BRAF/NRAS MAF alterations in the primary and metastatic tumors (brain, liver and lung) using Chromosome Analysis Suite 3.3. Our data indicate that the increased MAF in metastases of BRAF mutant tumors was most frequently accompanied by a single copy increase of BRAF (polysomy). However, in case of lung metastasis we have repeatedly detected true BRAF gene amplification. On the contrary, in NRAS mutant tumors the most frequent genetic alteration is LOH of various chromosomal regions, aspecially in case of the liver metastasis. However, NRAS gene was diploid in primary and metastatic tumors. In case of NRAS gene we have observed a 120000 kbp sized copy-neutral regional uniparental disomy. In LOH-tumors we have detected an NRAS-SNP. In one such LOH case in metastases NRAS MAF tripled. In the other LOH tumor, NRAS MAF decreased significantly. It is our hypothesis that this copy-neutral LOH lead to an accumulation of the NRAS-SNP.

Metastasis genes of melanoma

Genome–wide CNV analysis of 18 primary human melanoma cell lines derived from primary tumors and metastases have been performed looking for metastasis associated alterations. Beside the reported alterations (NEDD9, IL6, EGFR, BRAF, MYC, MMP16 and MMP9 amplifications and CDKN2A/B loss) 7q and 12q losses and gains of 5p and 8q have been observed. In invasive/metastatic cell lines gain in GDNF (chr 5p) GPAA1, PLEC and SHARPIN (8q) were found. Validation analysis identified PTPN12, ADAM22, FZD1 and SMURF1 as metastasis associated genes. (*Koroknai V et al. Mel Res, 2015*)

OPN. Data mining and metaanalysis of publicly available datasets of human melanoma expression patterns OPN was frequently detected as component of the metastatic gene signature. OPN is a matrix ligand the receptor of which are β3 integrins, known to be overexpressed in human melanomas. OPN expression was analysed in 93 human melanoma cases at protein levels using IHC and at mRNA levels by q-PCR. Higher than 50% of primary tumors overexpressed OPN at protein levels. OPN expression was found to be associated with higher T stage and metastatic disease establishing OPN as a negative prognostic factor of human melanoma. (*Kiss T et al. Tumor Biol 2015*)

AQP. Our previous expression array analysis identified several candidate metastasis associated genes overexpressed in metastatic human melanoma models including AQP1. We have tested the clinical significance of AQP1 protein expression in a metastatic/non-metastatic melanoma patient cohort of 78. Significantly higher protein expression was detected in the primary tumors of metastatic patients which was associated with lymphovascular invasion and BRAF mutant status. AQP1 positive tumors showed shorted PFS and OS suggesting the metastasis promoting role for AQP1 in human melanoma. (*Imredy et al. Mel Res 2016*)

CNV analysis of metastatic melanomas indicated copy-gains in the chromosome 7p where the AQP1 gene is located. To explore the significance of this finding, we studied the AQP1 protein expression in 67 metastatic melanoma patients using immunohistochemistry on FFPE tumor tissue samples. Primary tumor samples were acquired from consecutive patients with intra-cranial (IC) (n=44) and extra-cranial (EC) (n=23) metastases. We compared the AQP1 expression of the primary tumors giving IC and EC metastases, and the AQP1 expression of corresponding primary and metastatic tumor samples. Histological data were correlated with long term clinical follow up. Patients with IC metastases had lower overall survival (p=0.02) and significantly higher AQP1 expression in the primary tumor (median H-score= 250 vs. 140, p = 0.044) compared to patients in the EC metastasis group. On the other hand, AQP1 expression was significantly lower in the metastatic lesions, compared to the corresponding primary tumors (median H-score = 35 vs. 250 p = 0.01). We have found also that within the brain metastases, AQP1 protein expression was higher in the melanoma cells far away from the capillaries as compared to tumor cells adjacent to capillaries, indicating a potentially hypoxia-driven expression of AQP1. Based on these observations we assumed that AQP1 in human melanoma may serve as a brain-metastasis initiating gene at least in case of BRAF mutant melanoma. *(Imredy et al. Pathol Oncol Res,2019)*

CCL8/Stromal components. Using mouse-specific expression arrays we were looking for potential metastasis promoting stromal factors in experimental human melanoma models and found 5 among which CCL12 and its human homologue CCL8 was confirmed in the primary tumor's stromal cells and melanoma cells respectively. CCL8 was than proved to be a chemotactic factor for human melanoma cells. In a metastatic/non-metastatic melanoma patient cohort of 52 cases, increased CCL8 expression was detected selectively in the primary tumors of lung metastatic patients confirming the organ selective metastasis promoting role for CCL8. (*Barbai et al. Oncotarget, 2015*)

Genom-wide copy number variation analysis

We performed a genome-wide copy number variation (CNV) analysis on 12 primary and their 34 visceral metastatic samples using OncoScan- (on fresh frozen samples) or Cytoscan assay (formalin-fixed paraffin embedded samples). The 12 cases included 5 BRAF-, 4 double wt and 2 NRAS mutant cases.
Comparing the molecular variants of the primary tumors there was no statistically significant differences in CNV incidences.
According to of our comparative analysis, each melanoma metastatic site has its characteristic CNV pattern. In brain metastases 3q13.33, 9q22.32, 12p13.33, 12q11, 14q32.33, 16p13.3, 21q22.3, 22q11.22 loci, involving CD86, PTCH1, SYT10, POLR3K, RHBDF1, PRMT2 and IGLL5 genes were affected by copy number gain. In the 1p22.1, 3q26.1, 4q34.3 loci, including TGFBR3, BRDT and EPHX4 genes were lost in 50% of the examined brain metastases due to LOH. Frequent gains of 5p15.33-5p15.2; 5p14.3; 5p13.3; 5q31.1; 5q32; 20q13.33 chromosomal regions were detected in liver metastases, containing genes GRB10, PRAME, PTCH1, PLXNA4, RAC3 or SNAI2. Genes BRK1, FANCA, FBXO21, LCE1D, POLR2A, ZRANB3 located on 21q22.12-21q22.13 are seemed to be lost in liver metastasis, some of them belonging to the metastasis suppressor gene category. Approximately 40% of pulmonary metastasis genes on 1p36.32, 2q36.3, 3q13.33, 3q28, 4q13.2, 6p25.3, 7p12.1, 7p11.2, 8p23.2, 8p12, 8q11.21, 8q21.13, 14q32.33, 16p13.3, 17p11.2, 19p13.3, 20q13.12 and 22q11.2 loci showed extra copy numbers involving genes CD86,(also seen in brain mets) DAW1, EGFR, DUSP22, PAG1, RAI1, SNAI2 (seen in lung mets), whereas 1q21.3, 8p11.22, 12q23.3, 16q24.3, 19p13.2 regions were lost compared to metastasis from different sites containing DCTN2, FANCA, LCE1D (both occurring in liver mets as well) or MBD6 genes.

We analyzed the driver oncogene specific CNV landscape of visceral metastases, as well. Considering liver metastases, heterozygous loss was mainly characteristic for WT samples, whereas the proportion of LOH was much higher in *NRAS* mutant samples than *BRAF* mutant or WT ones. Regarding low and high copy number changes, there is a trend of decreasing from BRAF to NRAS to WT. If visceral localization was not taken into account, the proportion of heterozygous loss was significantly (p=0.032) higher in the triple wild type metastatic tumors. We also analyzed the copy number changes without distinguishing between different metastatic sites. Gains and losses in 6p, 8q and 11q were observed only in *BRAF* mutant samples. Gain in 1p, and the loss of chromosomal regions in 16p were characteristic to triple wild type tumors alone. *NRAS* mutants were enriched in genes with higher copy number in 2p, 2q, 15q, 16p, 18q, 18p, 19q and 22q, furthermore 1p and 17p losses could be found only in these cases. Only wild type tumors carried CNAs of several members of the PRAME gene family. In our dataset *PINK1, PAFAH2* were grouped with PRAME genes according to Toppfun database to negatively regulate apoptosis, while regulating cell proliferation in the opposite manner along with *SYF2, LIN28A.*

In BRAF mutant melanomas, we observed CN gain of class I members of the major histocompatibility complex (MHC I) and the histone coding genes responsible for DNA packaging during gene expression regulating chromatin modification. In our data set, several galectins were affected by LOH only in BRAF mutant metastases.

NRAS mutant tumors were characterized by the increased copy number of several genes responsible for drug metabolism, especially the phase II conjugation enhancers called UDP glucuronosyltransferases. Participants of interleukin-1 pathway (IL1A, IL36B, IL37, IL36A, IL1F10, IL36RN, IL36G) are also overrepresented in NRAS mutants.

When data were aggregated according to the mutation status and the localization of melanoma metastases, lung metastases were excluded from further analysis, because all lung tumors were BRAF mutant. Brain^{BRAF} represented specific copy number gains on only 8q, including important "historical" genes of DNA damage repair machinery (*PRKDC, RRM2B, UBE2V2, UBE2W*) and a key player of cell survival regulation, *YWHAZ*. Another gene should be mentioned here, *TERF1* is proven to be important in telomere maintenance and melanoma development as well. *NRAS* mutation bearing brain metastases were enriched exclusively in gains of chr2, 8p and 22q localized genes, such as FGF9/20 subfamily signaling. FGF9 regulates vascular development. Members (*ITGA4,-A6,-AV*) of the integrin gene family were also affected by copy number gains; also important indicators of tumor stromal influence on tumor cells in the same angiogenesis related manner. Genes altered by LOH in liver^{BRAF} metastases in chr2, chr 8p and 8q24.1 and other additional alterations on 17q, 20q were detected, too. Beside several histon genes, HLA genes are located in these regions. In liver^{NRAS} samples CNA enriched pathways were quite similar to the ones described above in connection with *NRAS* mutant without distinguishing between different metastatic sites. Plenty of drug metabolism related and IL-1 pathway included genes were gained. Exclusively, compared to *BRAF* mutant liver metastases TNF signaling (e.g *TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, CARD14, BAG4, IKBKB*) was also affected by copy number gains. Liver^{WT} metastases were different from others in their LOH landscape, regarding LOH of genes (e.g *IL-13, -9, -4, -3*) in 3q11.2, 5q31.1, 7q11.22 and 11p11.12 only.

Based on these data we can assume that it is feasible to find frequent organ metastasis-specific genetic alterations in melanoma suggesting that the organ-selectivity has genetic background. We are currently exploring this issue using NGS technology to further characterise if these genes are affected my mutations as well.

Target therapy and molecular predictors

Bisphosphonates inhibit the posttranslational modification of small GTPase proteins by blocking farnesyl diphosphate synthase, the key enzyme of the mevalonate pathway upstream of FT or GGT. This enzyme is responsible for the production of farnesyl

diphosphate and geranylgeranyl diphosphate. Zoledronic acid (ZA) is an aminobisphosphonate currently established in the treatment and prevention of osteoporosis and bone metastases. The potential antitumor effect of aminobisphosphonates is especially important in melanoma treatment as they may impair the posttranslational modification of RAS proteins including NRAS, a frequently mutated oncogene in melanoma. The influence of NRAS, BRAF and PTEN mutational status on the aminobisphophonate effect was studied in a panel of thirteen human melanoma cells. ZA treatment decreased cell viability in two NRAS mutant melanoma lines at relatively low doses. In contrast, the BRAF mutant and PTEN wild-type cell lines showed the lowest sensitivity. These findings suggest that benefit from prenylation inhibition in melanoma may be dependent on the type of driver oncogenes. (*Garay T et al. PlosOne, 2015*, *Timár et al. Cancer Metast Rev 2018*) BPH1222 is a novel lipophyllic ZA-analogue the anti-melanoma effects of which was tested in several cell lines indicating that most of them are more sensitive as compared to ZA. BPH inhibited the PI3K/AKT signaling inducing apoptosis which was dependent on Rheb. *(Rittler et al. In J Mol Sci, 2019*)
Mutant BRAF is valid target in melanoma but the clinical efficacy is uncomparable to immunotherapies due to short efficacy and frequent relapses. Our and others preliminary studies indicated that *EGFR* is overexpressed in some melanoma and are even mutated in extracellular domains. EGFR activation was found to be maintained even in BRAF or NRAS mutant human melanoma (*Garay et al Pathol Oncol Res,2015*) We have tested EGFR-kinase inhibitors in vitro and in vivo models and found that BRAF mutant human melanoma cell lines are sensitive to EGFR-TKIs which can even be combined with BRAF inhibitors to increase their efficacy. (*Kenessey et al. Melanoma Res2018*).Furthermore, we have found in BRAF inhibitor resistant melanoma cells derived from treated patients an increased motility which is paralleled by increased EGFR expression but decreased sensitivity to EGFR-kinase inhibitors. However, in these cells PDL1 overexpression is a hallmark, suggesting that upon resistance to BRAF inhibitors the switch to checkpoint inhibitors is feasible. (*Molnar E et al. Int J Mol Sci 2019*).
We have also tested the combination of BRAF inhibition with HDAC inhibition in vitro. We have found that one possible target of HDAC is the Ca-pump PMCA4b which is an oncosuppressor in melanoma. The combination of BRAF inhibition with HDAC inhibition increased the antitumoral effects BRAFi suggesting another interesting novel target therapy option (*Hegedus et al. Future Oncol 2017*).
Zinc as an essential trace metal is a ubiquitous component of various molecules of the cell. Studies indicated that it may modulate functions of various cancer cell types, and can even inhibit metastasis formation in experimental models. In melanoma, zinc was shown to affect melanin production and to induce apoptosis. Using BRAF-mutant human melanoma cell lines, we have tested the effects of ZnSO4 on cell proliferation, survival, migration as well as in vivo on experimental liver colony formation. We have found that ZnSO4 has antiproliferative and proapoptotic effects in vitro. In SCID mice intraperitoneal administration of ZnSO4 specifically inhibited liver colony formation without affecting primary tumor growth. To reveal the molecular mechanisms of action of zinc in human melanoma, we have tested mRNA expression of zinc finger transcription factors and found a strong inhibitory effect on HIF1α, as compared to WT1 whereas HIF2α and MTF1 expressions were unaffected. Immunohistochemical detection of HIF1α protein in liver metastases confirmed its decreased nuclear expression after in vivo $ZnSO_4$ treatment. These data indicate that in BRAF mutant human melanoma zinc administration may have an antimetastatic effect due to a selective downregulation of HIF1α. (*Burian et al. Pathol Oncol Res 2019)*

Expression of checkpoint inhibitory receptors and HLA in tumor cells is regulated by IFN-produced by activated T-cells in tumor microenvironment. This process requires expression of IFN receptor, activation of JAK/STAT signaling and interferon regulatory element (IRE)

genes including IRF1. In melanoma there was no association observed between the IFN pathway alterations and any of the molecular subclasses (BRAF/NRAS/KIT). In immunotherapy-resistant melanomas 75% contained genetic alterations affecting *IFN pathway genes* involving LOH of IFNGR1/2, IRF1 and JAK2 or amplification of IFN pathway inhibitors SOCS1 and PIAS4. (*Ladányi et al. Sem Canc Biol, 2019*). Accordingly, IFN resitance and its genetic determinants is key issue in metastatic melanoma therapy. We have established a model of human melanoma cell line from which IFN-sensitive and resistant clones were derived in vitro which preserved this biological feature in vivo as xenograft. We have established IFN-resistance signature in vivo using microarray approach. The validation of the gene signature involved q-PCR measurements. After bioinformatic analysis and validation sequences we have established a human melanoma IFN-resistance gene signature of 32 genes where only 15% was proved to have IFN-responsive elements (IRE) in their promoters (ISG12A, IRF2, PDE9A and WNT7A). The overwhelming majority of the genes of this signature was found to be upregulated (78%). Some members of this gene signature belong to purinergic Ca-channels, but their inhibitors had no effect on IFN-resistant melanoma cells. Other members involved in drug-resistance, but our preliminary data indicated no change in drug sensitivity of the IFN-resistant clones. We are currently testing the hypothesis of a connection between IFN-resistance and metastatic potential of human melanoma. (*Tímár J, Cancer Metastasis, Seefeld, 2019*)

Publications (grant was acknowledged)

1. **Tímár J,** Vizkeleti L, Dioma V, Barbai T, Rásó E. Genetic progression of malignant melanoma. Cancer Metastasis Rev 35:93-107,2016, IF 5.316, D1

2. Doma V, Barbai T, Beleaua MA, Kovalszky I, Raso E, **Tímár J**. KIT mutation incidence and pattern of melanoma in central east Europe. Pathol Oncol Res. 2019 Epub
3. Doma V, Kárpáti S, Rásó E, Barbai T, **Tímár J**. Dynamic and unpredictable changes in mutant allele fractions of BRAF and NRAS during visceral progression of cutaneous malignant melanoma. BMC Cancer 19:786,2019 IF 3.288, Q1

4. Koroknai V, Ecsedi S, Vizkeleti L, Kiss T, Szász I, Lukács A, Papp O, Ádány R, Balázs M. Genomic profiling of invasive melanoma cell lines by array comparative genomic hybridization. Melanoma Res 26:100-107,2016 IF2.219, Q1

5. Kiss T, Ecsedi S, Vizkeleti L, Koroknai V, Emri G, Kovács N, Ádány R, Balázs M. The role of osteopontin expression in melanoma progression. Tumor Biol 36:7841-7847,2015, IF2.926, Q2

6. Imredi E, Tóth B, Doma V, Barbai T, Rásó E, Kenessey I, **Tímár J**. Aquaporin1 protein expression is associated with BRAFV600 mutation and adverse prognosis in cutaneous melanoma. Melanoma Res 26:254-260,2016, IF 2.219, Q1
7. Imredi E, Liszkay G, Kenessey I, Plotár V, Gödény M, Tóth B, Fedorcsák I, **Tímár J**. Aquaporin 1 protein expression of the primary tumor may predict cerebral progression of cutaneous melanoma. Pathol Oncol Res, 2018, Epub IF 2.4 Q2

8. Barbai T, Fejős Zs, Puskas LG, **Tímár J**, Rásó E. The importance of microenvironment: the role of CCL8 in metastasis formation of melanoma. Oncotarget 6: 29111-29128,2015, IF 6.359, Q1

9. Garay T, Kenessey I, Molnár E, Juhász E, Réti A, László V, Rozsas A, Döme B, Berger W, Klepetko W, Tóvári J, **Tímár J**, Hegedüs B. Prenylation inhibition-indeced cell death in melanoma: reduced sensitivity in BRAF mutant/PTEN wild type melanoma cells. PlosOne 10:e0117021, 2015 (no grant acknowledged)

10. **Tímár J**, Hegedüs B, Rásó E. The role of lipid signaling in the progression of malignant melanoma. Cancer Metastasis Rev 37:245-255,2018, IF 6.081, D1

11. Rittler D, Baranyi M, Molnár E, Garay T, Jalsowsky I, Varga IK, Hegedüs B, Aigner C, Tóvári J, **Tímár J**, Hegedüs B. The antitumor effect of lipophyllic bisphosphonate BPH1222 in melanoma models: the role of the PI3K/AKT pathway and the small G protein Rheb. Int J Mol Sci 20:e4917,2019, IF4.183, Q2

12. Garay T, Molnár E, Juhász E, László V, Barbai T, Dobos J, Schlech K, Pirker C, Grusch M, Berger W, **Tímár J**, Hegedűs B. Sensitivity of melanoma cells to EGFR and FGFR activatzion but not inhibition is influenced by oncogenic BRAF and NRAS mutations. Pathol Oncol Res 21:957-968,2015 (no grant acknowledged)

13. Kenessey I, Kramer Z, István L, Cserepes MT, Garay T, Hegedüs B, Dobos J, **Tímár J**, Tóvári J. Inhibition of epidermal growth factor receptor improves antitumor efficacy of vemurafenib in BRAF-mutant human melanoma in preclinical model. Melanoma Res 28:536-546,2018, IF 3.135, Q1

14. Molnar E, Garay T, Donia M, Baranyi M, Rittler D, Berger W, **Tímar J**, Grusch M, Hegedűs B. Long term vemurafenib exposure induced alterations of cell phenotypes in melanoma: increased cell migration and its association with EGFR expression. Int J Mol Sci 20:E4484, 2019, IF4.183, Q2

15. Hegedüs L, Padányi R, Molnár J, Pászty K, Varga K, Kenessey I, Sárközy E, Wolf M, Grusch M, Hegyi Z, Homolya L, Aigner C, Garay T, Hegedüs B, **Tímár J**, Kállay E, Enyedi Á. Histone deacetylase inhibitor tretament increases the expression of the plasma memrane Ca pump PMCA4b and inhibits the migration of melanoma cells independent of ERK. Future Oncol 7:95, 2017, IF 2.369, Q1

16. Burian Z Ladányi A, Barbai T, Piurko V,Garay T, Rásó E**, Tímár J**. Selective inhibition of HIF1A expression by ZnSO4 has antitumoral effects in human melanoma. Pathol Oncol Res, 2019, Epub IF 2.4 Q2

17. Ladányi A, **Tímár J**. Immunologic and immunogenomic aspects of tumor progression. Sem Canc Biol, 2019 Epub, IF 9.658, D1

Presentations, abstracts:

Vizkeleti L, Papp O, Doma V, Reiniger L, Piurko V, **Timar J**: Driver-mutation specific CNV patterns of distant metastatic melanomas. The FEBS Journal 284 (Suppl. 1) (2017): 285. (P.4.1-097)

Papp O, Vizkeleti L, Doma V, Reiniger L, Kovacs A, Piurko V, **Timar J**: CNV patterns of distant metastatic melanomas revealed by aCGH. The FEBS Journal 284 (Suppl. 1) (2017): 285-6. (P.4.1-098)

Rásó E, Ladányi A, Barbai T, Puskás L, **Timár J**. Identification of genomic connections between IFN-resistance and metastatic potential of human melanoma. Cancer Metastasis Conference, Seefeld, Austria,2019