Novel *CHD5*, a tumor suppressor gene in neuroblastomas

Neuroblastoma, a tumor of the sympathetic nervous system, is the most common childhood extracranial solid tumor. The authors have identified different patterns of genetic change that underlie these disparate clinical behaviors.

One of the most characteristic genetic changes in neuroblastomas is deletion of the short arm of hromosome 1 (1p). The m*Chd5* as a gene that controls proliferation, apoptosis, and senescence via the p19Arf/p53 pathway.

CHD5 is the tumor suppressor gene deleted from the 1p36.31 SRD in neuroblastomas and may be particularly important for anchorage-independent growth.

High CHD5 expression was also strongly associated with favorable outcome.

Data suggest that CHD5 expression is a potent prognostic variable, that it likely plays a role in neuroblastoma pathogenesis, and that it may play a role even in some tumors without apparent 1p deletion. Studies also demonstrate that CHD5 expression is not just a surrogate marker for 1p deletion because it was by far most strongly associated with Outcome. The epigenetic mechanism could inactivate expression or lower it sufficiently to functionally silence the gene.

Also, *CHD5* could be re-expressed by growing these cells in 5-aza-2-deoxycytidine, so this approach may have utility in treating neuroblastomas with low or absent CHD5 expression.

REFERENCES

1 Brodeur GM, Maris JM. Neuroblastoma. In: Pizzo PA, Poplack DG, eds. Principles and Practice of Pediatric Oncology. 5th ed. Philadelphia, PA: Lippincott; 2006:933-70 .

2 Fujita T, Igarashi J, Okawa ER, Gotoh T, Manne J, Kolla V, et al. *CHD5*, a Tumor Suppressor Gene Deleted From 1p36.31 in Neuroblastomas. *J Natl Cancer Inst.* 2008;100:940–9.

3 Bagchi A , Papazoglu C, Wu Y, Capurso D, Brodt M, Francis D, et al . CHD5 is a tumor suppressor at human 1p36. *Cell.* 2007;128(3):459–75.

Vladimir Baltić

Tumor-associated trypsin inhibitor (TATI) in human colon cancer cells

The human colon carcinoma cells, HT-29 5M21 (CM-5M21), expressing a spontaneous invasive phenotype, tumor-associated trypsin inhibitor (TATI). Both CM-5M21 and recombinant TATI, trigger collagen type I invasion by several human adenoma and carcinoma cells of the colon and breast, through phosphoinositide-3-kinase, protein kinase C and Rho-GTPases/Rho kinase-dependent pathways.

Stable expression of K18Y-TATI in HT-29 5M21 cells downregulated tumor growth, angiogenesis and the expression of several metastasisrelated genes, including CSPG4, BMP-7, the BMP antagonist CHORDIN, IGFBP-2 and IGF2.

TATI inhibits trypsin with strong affinity, as well as plasmin and urokinase, however with lower affinities. This inhibition is reversible since the complex is dissociated by serum. TATI is inactivated by trypsin cleavage between Arg 42-Lys 43 and Arg 44-Gln 45.

TATI has been originally found in urine of a patient with ovarian cancer. The high levels of TATI in tumors and/or body fluids were reported in cancers of pancreas, colon, liver, lung and breast.

TATI may participate at the signaltrans duction mechanisms involved at the adenoma–carcinoma transition during the neoplastic progression in colon cancer patients. The TATI is a permissive factor for tumor growth and cell survival.

Increased levels of trypsinogen, tumor-associated trypsinogens, trypsin and its inhibitor TATI correlate with the malignancy of human solid tumors. The presence of TATI in tumors is a marker of adverse prognosis for hepatocellular carcinoma (HCC), bladder, kidney and mucinous ovarian cancers. Increased TATI serum levels occur in 34–74% of patients with colon cancer.

The mechanisms underlying TATI overexpression in human tumors and its accumulation in the serum of cancer patients are largely unknown. This induction can be accomplished via transcriptional activation of the TATI gene promoter and epigenetic regulations, including alternative DNA methylation states.

These findings identify TATI as an autocrine transforming factor potentially involved in early and late events of colon cancer progression,incl uding local invasion of the primary tumor and its metastatic spread. Targeting TATI, its molecular partners and effectors may bring novel therapeutic applications for high-grade human solid tumors in the digestive and urogenital systems.

REFERENCES

1 Paju A, Stenman UH. Biochemistry and clinical role of trypsinogens and pancreatic secretory trypsin inhibitor. *Crit Rev Clin Lab Sci.* 2006;43:103–42.

2 Kotelevets L, Noë V, Bruyneel E, Myssiakine E, Chastre E, Mareel M, et al. Inhibition by platelet-activating factor of Src- and hepatocyte growth factor-dependent invasiveness of intestinaland kidney epithelial cells. Phosphatidylinositol 30-kinase is a critical mediator of tumor invasion. *J Biol Chem.* 1998;273:14138–45.

3 Nguyen QD, De Wever O, Bruyneel E, Hendrix A, Xie WZ, Lombet A, et al. Communtators of PAR-1 signaling in cancer cell invasion revealan essential role of the Rho-Rho kinase axis and tumor microenvironment. *Oncogene.* 2005;24:8240–51.

4 Lee YC, Pan HW, Penq SY, Lai PL, Kuo WS, Ou YH, et al. Overexpression of tumour-associated trypsin inhibitor (TATI) enhances tumour growth and is associated with portalvein invasion, early recurrence and a stage-independent prognostic factor of hepatocellular carcinoma. *Eur J Cancer.* 2007;43:736–44.

5 Stenman UH. Tumor-associated trypsin inhibitor. *Clin Chem*. 2002;48:1206–9.

6 Gouyer V, Fontaine D, Dumont P, de Wever O, Fontayne-Devaud H, Leteurtre E, et al. Autocrine induction of invasion and metastasis by tumor-associated trypsin inhibitor in human colon cancer cells. *Oncogene.* 2008;27:4024–33.

Vladimir Baltić

SATB1 reprogrammes gene expression to promote breast tumor growth and metastasis

The ability to treat tumors by targeting epigenetic processes is fast becoming reality. In a recent issue of *Nature*, Han et al. described a new chromatin-organizing protein associated with the progression of breast cancer.

Spatial AT-rich-binding protein 1 (SATB1) is a genome organizer that tethers multiple genomic loci and recruits chromatin-remodelling enzymes to regulate chromatin structure and gene expression. SATB1 promotes a transcriptionally active chromatin structure by interacting with AT-rich DNA sequences that tend to become unpaired under conditions of torsion stress, and induce loop formation. SATB1 regulates histone modifications and nucleosome positioning over long stretches of DNA.

SATB1 is expressed by aggressive breast cancer cells and its expression level has high prognostic significance, independent of lymph-node status. In primary breast tumors SATB1 protein was detected in all of the poorly differentiated infiltrating ductal carcinomas tested and majority of moderately

differentiated samples, but not in the surrounding normal tissue. Assessment of 1.318 evaluable breast cancer specimens established a strong correlation ($P < 0.0001$) between high SATB1 protein and shorter overall survival time.

Genetic knockdown of SATB1 by short hairpin RNAs (shRNAs) in two highly aggressive breast cancer cell lines (MDA-MB-231 and BT549) altered the expression of > 1.000 genes reversing tumorigenesis by restoring breast-like acinar polarity and inhibiting anchorage-independent tumour growth and metastasis *in vivo*. SATB1 delineates specific epigenetic modifications at target gene loci, directly upregulating metastasis-associated genes while downregulating tumour-suppressor genes. SATB1 reprogrammes chromatin organization and the transcription profiles of breast tumours to promote growth and metastasis; this is a new mechanism of tumour progression.

The authors propose that SATB1 may be usefull therapeutic target in metastatic breast cancer. These findings suggest novel approaches to preventing cancer cell invasiveness and improving the reliability of prognostic tests for breast cancer.

The approval of azacitidine (Vidaza) in 2004 and decitabine (Dacogen) in 2006 for the treatmen of myelodysplastic syndrome demonstrated that DNA hypomethylating agents can provide patient benefit with acceptable toxicity. Verinostat (Zolinza), a histone deacetylase inhibitor, was approved in 2006 for the treatment of cutaneous manifestations of refractory cutaneous T-cell lymphoma, and several other histone deacetylase inhibitors in clinical development show promising activity on selected cancers, especially when combined with other drugs.

The identification of a new epigenetic mechanism linked to breast cancer highlights the promise of the epigenome as an exciting area for cancer drug discovery.

REFERENCE

1 Han H-Y, Russo J, Kohwi Y, Kohwi-Shigematsu T. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. *Nature*. 2008;452:187-93.
2 Richon VM. A new path to the cancer epigenome. *Nat Biotechnol*. 2008;26(6):655-6.

Dragana Četojević-Simin

Rituximab maintenance therapy in follicular lymphoma

Follicular non-Hodgkin's lymphomas (FL) are generally associated with long survival, measured in years even if initially untreated, but they are usually not curable with conventional treatment (1). Many randomised trials showed increases in response rate, response duration and overall survival, without increase in toxicity after addition of rituximab (R) to chemotherapy (1). After those results many scientific groups try to prolong remission with R maintenance. The Swiss group for clinical cancer research (SAKK) study (2) showed overall response rate (OS) 45% vs. 28 % complete response rate (CR) of 29 vs 19% and progression free survival (PFS) of 23.2 vs. 11.8 ($p=0.02$) months in patients receiving R maintenance vs. observation respectively. In this study patients were untreated or pretreated and receive induction with R monotherapy. In Hainsworth study (3) pretreated patients received R induction and randomized to receive R maintenance or observation. PFS was greater after R maintenance 31.3. vs 7.4 ($p=0.007$) months. European Organisation for Research and Treatment of Cancer (EORTC) study (4) randomize patients to receive R maintenance vs. observation after initial treatment with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) or R-CHOP in relapsed or resistant FL. Patients who received R maintenance after CHOP and R-CHOP had significantly longer PFS. 42.2 vs 11.6 ($p < 0.001$) in CHOP induction arm and 51.8 vs 23 ($p < 0.001$) months in R-CHOP induction arm. Similar study was performed by Forstpointner et al. on behalf of German Low-Grade Lymphoma Study Group (GLGS) (5). Patients were randomized to receive FCM (fludarabine, cyclophosphamide, mitoxantrone) or R-FCM for relapsed or resistant FL. After induction randomization was between R maintenance and observation arm. Both treatment showed statistically significant longer PFS with R maintenance ($p < 0.01$) and borderline statistically significant overall survival (OS) (82 vs 55% at 3 years). Serious infection rate was similar in both arms ($p = 0.72$). Similar results in terms of PFS and OS was in Hochster et al. (6). Patients were treatment naive and receive CVP chemo induction therapy. Hochster study is only trial with maintenance after first line treatment. As a conclusion, rituximab maintenance showed benefit in progression free survival in four trials for relapsed or resistant FL no matter of usage of R in induction. Two questions are still opened: 1) which treatment schedule for maintenance is optimal and 2) what is the role of prolong rituximab treatment after, nowadays, golden standard of first line treatment, R-Chemo. Some of the answers will be provided after the termination of PRIMA study. R maintenance should be considered as very important part of FL treatment.

REFERENCES

1 Forstpointner R, et al. *Curr Hematol Malign Reports*. 2007;2:213-8.
2 Ghielmini M, et al. *Blood*. 2004;103:4416-23.
3 Hainsworth JD, et al. *J Clin Oncol*. 2005;23:1088-95.
4 van Oers MH, et al. *Blood*. 2006;108:3295-301.
5 Forstponitner R, et al. *Blood*. 2006;108:4003-8.
6 Hochster H, et al. *Blood*. 2005;106: Abstract 349.

Lazar Popović

Anti-CD20 MAB(s)..., but not ...THERA

CD20 is a membrane antigen expressed generally in B-cell lymphomas. Rituximab (Mabthera, Roche) is the first antibody approved by FDA in 1997. It is chimerical antibody that targets CD20 antigen and has become the golden standard for the treatment for many different types of B-cell non-Hodgkin's lymphomas (1). Rituximab action includes few different mechanisms. Complement-dependent cytotoxicity (CDC) is the most important. Other mechanisms are antibody dependent cytotoxicity (ADCC), direct cytotoxicity though induction of target cell signalling and vaccine-like effect (2). Because of its chimerical (murine) fragment the most common adverse event of rituximab is infusion reaction with symptoms like chills, fever, and hypotension (1). Several novel "second generation" of anti-CD20 antibodies are undergoing preclinical and clinical development (2). Ofatumumab (HuMax-CD20, GlaxoSmithKline) is fully human IgG1 antibody that has increased CDC activity relative to that of rituximab (2). The reason for enhanced CDC activity is that ofatumumab is binding to an epitope on CD20 closer to cell membrane and has a slower off rate. Its action is independent of expression of complement inhibitory proteins CD55 and CD59 (3). Ofatumumab was tested in I/II phase clinical trial in subjects with relapsed follicular lymphoma. Toxicity was similar to that of rituximab and responses occurred in 15 of 36 patients including 8 of 14 previously treated with rituximab. An area of focus for this agent has been in chronic lymphocytic leukemia (CLL).

In a phase I/II trial response rate was about 50% (2). Another novel anti-CD20 antibody is veltuzumab (hA20, Immunomedics). It is humanized antibody that delivered objective responses in indolent lymphomas (phase I/II) in doses lower than those generally used with rituximab (2). Since ADCC is important mechanism of action for rituximab, numerous efforts are attempting to enhance antibody binding to activating Fc receptors to potentially improve potency. GA101 (Roche) is type II antibody with greater ADCC and apoptosis activity compared to that of rituximab. Ongoing clinical studies will determine whether these modifications result in improved efficacy. AME-133 (AME) is human IgG1 antibody with Fc region capable of binding to CD16 with a 5 to 10-fold greater affinity than rituximab. That is the reason why AME-133 was more effective than rituximab at natural-killer cells activation and can overcome the effects of less favourable Fc receptor polymorphisms (2). Beside those "naked" antibodies, there also antibodies with the radioisotopes: Y90-ibritumomab-tiuxetan (Zevalin, Bayer Schering Pharma) and I131-tositumomab (Bexxar, GlaxoSmithKline). Zevalin is an anti-CD20 antibody with β-emitter Yttrium-90 and Bexxar is an antibody with β and γ emitter Iodine-131. They're both approved for the treatment of different types of CD20-positive non-Hodgkin lymphomas (1). Whether those novel antibodies will enter everyday clinical practise depends on phase III trials. They have to beat rituximab "one-on-one", or to be good alternative for rituximab refractory patients.

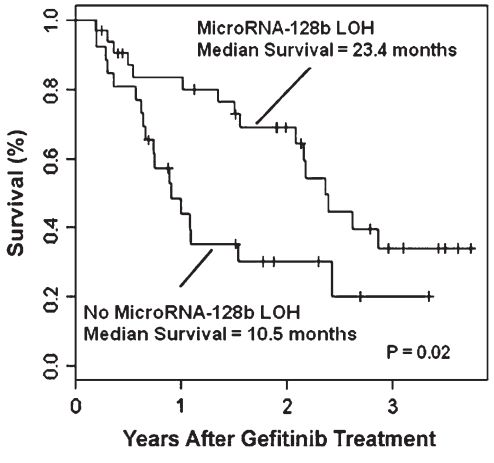
REFERENCES

1 Fanale M, et al. *Drugs*. 2007;67(3):333-50.
2 Leonard JP, et al. *Ann Oncol*. 2008;19 Supp 4:61-5.

Lazar Popović

microRNA-128b correlation with clinical response and EGFR expression

The EGFR is a potential target of microRNA-128b, which located on chromosome 3p22. Allelic loss on chromosome 3p22 is one of the most frequent and earliest genetic events in lung carcinogenesis (1). The EGFR is a poor predictor of survival patients receiving treatment with EGFR-TKIs, then patients with EGFR mutation (EGFR exons 18-21) or gene amplification (2).



No. of Patients at risk:

MicroRNA-128b LOH	*32*	*24*	*15*	*7*
No MicroRNA-128b LOH	**26**	**10**	**5**	**3**

Figure 1. Effect of microRNA-128b loss of heterozygosity on survival (3)

The microRNA-128b LOH identification is adequate additional predictor factor which correlated significantly with clinical response and survival following gefitinib. EGFR expression and mutation status did not correlate with survival outcome (Figure 1) (3).

These investigations support the contention that microRNA regulation could have far-reaching implications for lung cancer patients, including screening for selection of patients to receive targeted agents, or development of early biomarkers of lung cancer.

REFERENCES

1 Wistuba II, Behrens C, Virmani AK, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. *Cancer Res*. 2000;60:1949–60.
2 Dziadziuszko R, Hirsch FR, Varella-Garcia M, et al. Selecting lung cancer patients for treatment with epidermal growth factor receptor tyrosine kinase inhibitors by immunohistochemistry and fluorescence in situ ybridization—why, when, and how? *Clin Cancer Res*. 2006;12:4409s–4415s.
3 Weiss GJ, Bemis LT, Nakajima E, et al. EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. *Ann Oncol*. 2008;19(6):1053-9.

Vladimir Baltić
Milan Baltić

Female sexual dysfunction after extended pelvic suregery procedures in the treatment of malignant diseases

The sexual response cycle in men and women includes the basic stages of sexual desire, arousal and orgasm. Female sexual dysfunction does not exist as a single diagnosis, it consists of specific disorders which are classified such as female arousal disorder, female desire disorder, female orgasmic disorder and sexual pain. One of them or their combination result in significant personal distress and may have a negative effect on a woman's health and an impact on the quality of life. Etiology of female sexual dysfunction is complex but some of causes are observed as very important like psyhogenic factors, neurological disorders, endocrine alterations, cerebrovascular dysfunctions and state after pelvic surgery especially in the treatment of malignant diseases.

When a conventional low anterior and abdomino-perineal resection with extended lymphadenectomy is performed for advanced lower rectal cancer, sexual and bladder functions are often sacrificed reported to be between 10% and 60% (1). Sexual and bladder dysfunctions are usually caused by a non-nerve sparing surgical approach with the damage of one or more of the autonomic nerves consisting of the paired sympathetic hypogastric nerve, sacral splanchnic nerves and the pelvic autonomic nerve plexus. Multimodality treatment (surgery in combination with radiotherapy) can increase the chances of damaging the urogenital nerves and organs which could result in voiding and sexual disorders (2). Mannaerts et al reported the sexual outcome results of women suffering from locally advanced rectal cancer after aggressive multimodality therapy. The results confirm that interest in sexual activity and ability to achieve orgasm decreased in those women. 56% of respondants complained of sexual inactivity. Despite urinary and sexual dysfunction, most patients were satisfied with their quality of life (3).

Genitourinary cancers are commonly associated with treatment-related sexual dysfunction varying from mild to severe. Women undergoing cystectomy with simultaneous removal of uterus, ovaries, and parts of the vaginal wall face had issues regarding their femininity as well as concerns regarding future sexual function (4). Urinary diversion provides a better quality of life with maintenance of sexual function and urinary continence (5). Compared with women with bladder cancer, those with incontinence/bladder dysfunction were more likely to have an active sexual life after urostomic surgery (6). Horenblas et al reported preliminary results of a modified cystectomy called sexuality preserving cystectomy and neobladder, the intent of the surgical technique being to achieve maximal tissue conservation and potentially preserving normal sexual function and satisfactory urinary tract reconstruction. Three women aged 38 to 71 years old were enrolled in this protocol and all reported normal vaginal lubrication during sexual activity (7).

Reports of deterioration of sexual function after hysterectomy are estimated to be between 13% and 37%. Quality of sexual life after hysterectomy may be influenced by several situations. In a comprehensive review article, Carlson reported that women undergoing hysterectomy for nonmalignant conditions there is a marked improvement in symptoms and quality of life during the early years after surgery (8). The authors showed that both sexual desire and frequency of sexual relations significantly increased after hysterectomy such as great improvement of vaginal dryness. In contrast Rako reported a decrease in quality and frequency of sexual activity after hysterectomy emphasized the importance of the ovaries as a critical source of testosterone as well as estrogen. Thus removal of the uterus, even after ovary-sparing procedures, can jeopardize their function. Loss of a physiologic level of testosterone, estrogen and progesteron in women after hysterectomy can decrease quality of life in terms of libido, sexual pleasure and sense of well being (9). These conditions are made worse when hysterectomy is accompanied by bilateral oophorectomy. The estrogen deficiency is often associated with vaginal dryness and testosterone decreased level with lack of libido. The vaginal orgasm should be hindered by hysterectomy with cervix removal because of damaged uterovaginal plexus but theoretically clitoral orgasm should not be disturbed. However, surgical damage to the pelvic autonomic nerves during radical hysterectomy is thought to be responsible for considerable morbidity, including sexual dysfunction. Surgical preservation of the pelvic autonomic nerves in both laparoscopic and traditional radical hysterectomy deserves consideration in an attempt to improve both cure and quality of life in cervical cancer patients (10).

REFERENCE

1 Havenga K, Deruiter MC, Enker WA, Welvaart K. Anatomical bases of autonomic nerve preserving total mesorectal excision for mesorectal cancer. *Br J Surg*. 1996;83:384-8.

2 Hashiguchi Y, Sekine T, Skamoto H, Tanaka Y, Kazumoto T, Kato S, et al. Intraoperativne irradiation after surgery for locally advanced rectal and rectosigmoid carcinoma. *Dis Colon Rect.* 1999;42:886-95.

3 Mannaerts GHH, Schjiven MP, Hendrix A, Martijin H, Ruten HJT, Wiggers T. Urologic and sexual morbidity following multimodal treatment for locally advanced primary and locally recurrent rectal cancer. *EJSO*. 2001;27:265-72.

4 Marshall FF, Treiger BF. Radical cystectomy (anterior exenteration) in the female patient. *Uro Clin North Am*. 1991;18:766-75.

5 Bjerre BD, Johansen C, Steven K. A questionnaire study of sexological problems following urinary diversion. *Scand J Urol Nephrol*. 1997;31:155-60.

6 Hautman RE, Paiss T, De Petriconi R. The ileal neobladder in women: 9 years of experience with 18 patients. *J Urol*. 1996;155:76-81.

7 Horenblas S, Meinhardt W, Ijzerman W, Moonen LF. Sexuality preserving cystectomy and neobladder: initial results. *J Urol*. 2001;166:837-40.

8 Rhodes JC, Kjerulff KH, Langenberg PW, Guzinski GM. Hysterectomy and sexual function. *JAMA*. 1999;282:1934-41.

9 Rako S. Testosterone supplemental therapy after hysterectomy with or without oophorectomy : estrogen alone is not enough. *J Womens Health*. 2000;9:917-23.

10 Quereley D, Narducci F, Poulard V, lacaze S, Occeli B, Leblence E, et al. Modified radical vaginal hysterectomy with or without nerve spering dissection: a comparative study. *Gynecol Oncol*. 2002;85:154-8.

Dejan Ninčić