Romanian researchers and cancer genetics: where are we leading to?

Author: Ana-Sonia Ignat

ckground

Aiming to evaluate the genetic risk factors associated with breast cancer (in female), colon and rectum, prostate and lung cancer, the ‘ROMCAN’ project needs a review of the latest publications, targeting genetics in these type of cancers, also assessing the Romanian researchers’ activity in this field.

otocol:

eview question and inclusion criteria:

- Q: Who is targeted? A: Romanian researchers
- Q: What is the field of interest? A:Genetics in female breast cancer, colon and rectum, prostate and lung cancer
- Q: When were the articles published? A: Article date from the last five years

tudy inclusion and exclusion criteria:

- Population => general population affected by breast cancer (females), colon and rectum, prostate and lung cancer
- Intervention and comparators =>genotyping
- Outcomes => the influence of a genetic mutation on the diagnosis and prognosis of the targeted malignancies
- Study design => comparative studies of any design assessing the mutational status' influence on diagnosis and prognosis of the targeted malignancies

  -original articles

  -reviews

Breast cancer

reast cancer

ncreasing incidence and mortality, especially in the underdeveloped countries

lowadays, a particular importance: given to the molecular biology and mmunohistochemistry

ole of immunohistochemistry in breast cancer's pathology:

- Possibility to characterize different types of tumors
- Confirmation of the tissue origin
- Differentiation of the metastasis from the primary tumor
- Role in dictating the response to treatment, the prognosis, or to identify an eventual tumor after the treatment

aily issues in breast tumor pathology:

- Precise differentiation of various breast benign tumors of carcinoma
- Differentiation of carcinoma in situ from invasive carcinoma
- Accurate diagnosis of the breast as primary site in metastatic carcinoma

east cancer:

Mammary histology and the normal expression of certain proteins:

breast tissue-composed of **3 types of cells**: luminal, basal and myoepithelial

Luminal cells express: cytokeratins (CK 7, 8, 18, 19), epithelial membrane antigen (EMA), α-lactalbumin, estrogen receptor (ER), progesterone receptor (PR)

Myoepithelial cells express: cytokeratin type basal cells, smooth muscle actin type marker, calponin, S100, p63

Basal cells express: cytokeratins (5/6, 14, 17)

east cancer:

***Diagnostic markers**:*

- **Myoepithelial markers**: used in differentiating the invasive carcinoma from benign proliferations with similar morphology
- For the evaluation of intraductal proliferative lesions, cytokeratins with high molecular weight (14, 5/6) can be used= › differentiation of ductal hyperplasia from poorly differentiated carcinoma in situ (DCIS)
- Athypical ductal hyperplasia or carcinoma in situ may occur in benign papillary lesions= defined as a type of ductal hyperplasia that stimulates DCIS=› the need of immunohistochemistry for its differentiation

Smooth muscle actin (SMA): often used in pathology, as myoepithelial marker, although there is he tendency of replacing it , due to its lack of specificity

Eremia et al. uses IHC markers in evaluating 9 cases of invasive papillary carcinomas

- The difference between a benign papillary lesion and carcinoma (invasiveness study) was made based on the presence/absence of the myoepithelial layer
- The reactivity for alpha SMA was tested
- CK 5/6 – rated as present in the basal membrane, focal positive in ductal hyperplasia, partially absent in atipic hyperplasia and negative in carcinomas
- p63 – strongly positive in myoepithelial cells, absent in stroma and in the epithelial cells of the benign papillary lesions
- Results: the importance of IHC use in differentiating the benign lesions from the malignant and taking therapeutic decisions, in case of papillary breast lesions.

east cancer:

63- location at nuclear level and the expression exclusively at the level of myoepithelial cells f the normal breast tissue=› specificity of almost 100%

-cadherin: modulates the mechanism of tumoral invasion, either through individual articipation or through creation of certain complexes with other family members of adherins

- Classification:
  - classic cadherins – part of the epithelial belt of adhesion
  - non-classic cadherins – part of desmosomes = desmocollin and desmoglein
- E-cadherin: tumor suppressor gene
- Key elements in the epithelial-mesenchymal transition mechanism
- Loss of expression: can be attributed to genetical alterations

. Ionescu Popescu et al.- the profile of **E-cadherin** in the different molecular subtypes of reast cancer=› a possible different expression according to this

ossible correlations between E-cadherin, molecular and clinical-pathological aspects

esults: prove the presence of a high expression in 26 cases, the rest of 16 presenting a low xpression

-cadherin- preognostic factor, through the connection from a low expression of this with a igh tumoral degree

east cancer:

-cadherin: also evaluated by **C.E. Cotrutz et al**. in a study focused on emonstrating the presence of E-cadherin in 34 cases of ductal invasive breast arcinoma, metastatic or not, using IHC and TEM (transmission electron nicroscopy)

esults: an association of DIC with a low differentiation degree and high tumoral egree, as also complete desmosomal integrity in non-metastatic DIC

Veak correlation between the nodular-lymphocyte status and the presence of E-adherin

Connection between the expression of E-cadherin and observed desmosomal ltrastructural modifications, regarding the invasive character= prognostic factor?

nterleukin 6 -IL6: antiapoptotic key factor involved also in promoting cell roliferation

- Controversial rose in tumorigenesis
- Found in the serum of patients with different tumors, including breast cancer

east cancer:

CCL18: -controversial role in cancer

- Excessive production at the level of M2 macrophages was associated with chronic inflammatory diseases and tumors
- **D. Narița et al.** propose the quantification of CCL18 and IL-6 in different breast tumors, with the purpose of evaluating their role in the diagnosis, evaluation and probably therapy of breast cancer
- Results: both tested markers were up-regulated in the case of cancers in comparison with benign tumors or control cases
- An up-regulation to the limit of significance for CCL18 regarding the cancer in comparison with the benign tumors or control cases
- No statistically significant differences were obtained in case of IL-6, regarding the comparison cancer-benign/control tumors
- Increased expression of IL-6 appears to confer a weak prognosis to the ER+ cancers
- CCL18= associated with other indicator factors of a poor prognosis, such as Ki-67.

reast cancer:

Prognostic markers

Hormone receptors for estrogen (ER) and **progesterone** (PR) represent biomarkers with prognostic and predictive role in modulating the treatment of breast carcinoma

ER: 2 types of receptors, **ER alpha** and **ER beta**

The progesterone receptors belong to the same class of nuclear receptors, being classified in 2 forms, **B** and **A**

M. Moise et al: study on over 80 breast carcinomas

- Correlation between the ER and PR expression with clinic-pathological parameters recognized as prognostic factors: menopausal status, tumor staging, its size, lymph nodes status, degree of differentiation, histological type
- Results: the hormonal fond of development and progression of breast tumor gland was found to be strongly interconnected with the estroprogestative profile.
- Testifies the presence of metatstatic tumors which do not express ER/PR
- Presence of different ER/PR tumoral phenotype
- The need of continuous research in the treatment of breast cancer

east cancer:

.M. Plesan et al. study the ER/PR expression, taking into consideration only their nuclear xpression

- Results: improved prognostic value of the expression of hormonal receptors, by ER/PR correlated evaluation
- Usefulness in identification of tumors with a heterogeneous phenotype

Ier2/neu: the mutant analog of the receptor for EGF (epithelium growth factor), is an ncoprotein involved in the control of normal cellular growth as in the normal cellular ivision

he oncogene (also known under the name of neu, HER-2, c-erbB-2 and EGFR2): in direct orrelation with the tumoral agressivity and chemoresistance

Ier2/neu status is a prognostic factor of the response at the therapy with trastuzumab

. Recăreanu et al. realize the analysis of 75 ductal invasive breast carcinomas, focusing on he comparative study of the hormonal receptors and the expression of Her2/neu oncoprotein

esults: the expression of the receptors ER and PR is inversely correlated with the Her2/neu xpression

he immunophenotype expressing ER+/PR+ had the highest incidence, while the mmunophenotype ER-/PR+ had the lowest incidence

he importance of an individualized therapy

east cancer:

N. Pătrană et al evaluated the predictive role of Her2/neu and its influence over the esponse to antitumoral treatment

- Results: negative expression of Her2/neu in case of most invasive breast carcinomas
- Positive expression correlated especially with the age (appears at young people)
- Large tumoral dimensions, reduced differentiation degree (G3), increased number of involved lymph nodes= › a poor prognosis to these patients

Similar results obtain also **M. Moise et al**., in their study in which evaluate the verexpression Her2/neu in breast tumors

A. Belengeanu et al. use both IHC (immunohistochemistry) and FISH (fluorescent in situ ybridization) in order to evaluate the Her2 overexpression in breast carcinomas

Results: similar with the other studies, which place Her2 among the prognostic factors

mportance of evaluation Her2 status and hormonal status before beginning the therapy

east cancer:

he existence of studies which already testify the **rezistance to the trastuzumab** therapy, onsidered **in the context of losing the expression for PTEN gene** which =› hormonal herapy might not influence the prognosis on long term

cute need of identifying new therapeutic targets

EGFR: proven role in molecular diagnosis of breast carcinoma

- Important role also in the rezistance to chemo-radiotherapy treatment=› poor prognosis
- Its expression in all molecular subtypes and correlations with classic clinic-pathological predictive factors, was evaluated by **A. Meche et al**
- Results: a positive EGFR reaction limited to the basal-like subtypes, HER2 and luminal B, which covers 2 forms – membrane – restricted pattern and abberant expression
- Correlations between EGFR, tumoral stage, histological subtype and differentiation degree were not found

nteresting correlations, between the mammographic aspects and oncoproteic status Her2/ eu, realize **D. E. Enache et al.**in their research which follows the evaluation of ER- nvasive breast carcinomas.

east cancer:

he **triple-negative tumors** : ER negative, PR negative, HER2 egative phenotype

- 10-20% of all breast tumors, associated with a poor prognosis and with an extremely aggressive behavior – diagnosis at young age, high tumoral degree, large dimensions of the primary tumor, aggressive recurrents.
- No specific treatment

ancer stem cells (CSCs): unknown origin

- Have the properties of certain real stem cells, disposing of replication capacity and high proliferation rate
- Found in hematologic disorders as in a series of solid malignant tumors, including breast cancer
- CD44+CD24- expression

east cancer:

M. Comănescu and G. Bussolati evaluate CSC in the context of riple-negative invasive carcinomas, suggesting the need of reatments targeted against these types of cells

- Recognition of **CD44+CD24-** as valuable **marker of cancer stem cells**

east cancer:

Apoptotic markers and of cellular proliferation

Ki-67: nuclear protein normally expressed in G1 phase of the cellular cycle

- A useful marker of cellular proliferation
- **D.M. Pleșan et al**. evaluate the expression IHC of Ki-67 on 100 breast carcinomas, 98% of them expressing Ki-67 at nuclear level
- The same study also evaluates the p53 expression, whose intracellular growth appears to be connected, in certain tumors, with a poor prognosis and a weak response to treatment

east cancer:

P53 is an intense studied marker in cancer, but its predictive value is still contested

- The suppressor gene p53 encodes a protein involved in the control of cellular proliferation
- P53 immunopositivity is associated with a poor prognosis, associating an increased tumoral degree, high cellular proliferation rate and an aggressive behavior

Apoptosis, programmed cellular death, is a process controlled by a number of factors inducers) intra and extra cellular, that can affect positively or negatively this process

Bcl-2 belong to a family of Bcl proteins that regulates the apoptosis, Bcl-2 gene being involved in the etiopathogenesis of a large number of malignant tumors.

- Considered a factor of rezistance to conventional anti-tumoral treatments
- A research of the apoptosis in breast cancer, through the spectrum of the Bcl-2 expression, was made by **D.C. Haza and E. Lazăr**
- Results: the results of the evaluation of 61 cases highlight the positivity for Bcl-2 in 54% of cases, with significant statistic differences regarding the hormonal status
- Regarding the association with tumoral dimension, was observed positivity for Bcl-2 in case of tumors of small dimensions, and negativity for Bcl-2 for tumors of large dimensions.

east cancer:

The family of claudins is composed of 24 transmembrane proteins which express ransmembrane specificity, joining the composition of endothelial and epithelial unctions (TJ).

- These proteins are strictly necessary in the adjustment of the cellular cycle, cellular differentiation, polarity, epithelial subdivision.
- There is data that testifies the involvement of these in carcinogenesis, through the TJs structure degradation and alteration of their function

Ionescu Popescu at al. evaluates the distribution of claudins at tumoral level

- The present study evaluated the expression CLDN3, expression correlated also with the hormonal status, 5 from the 9 cases being ER+, the rest ER-
- CLDN3 in phenotype ER- indicates the fact that the absence, or weak expression, is most commonly associated with basal-like or normal-like subtypes

CLDN= a reliable marker of the endothelial and epithelial junction integrity, frequently associated with the luminal subtype, in case of breast carcinomas

east cancer:

Iammaglobin: a glycoprotein ussually expressed by the norrmal breast tissue, verexpressed in breat cancer.

- An attractive target in anti-tumor therapy research

I.Raica et al assessed mammaglobin A's expression in breast cancer

- Results: marker of good prognosis

iven the fact that not much is known about the profile of lymphangiogensis in uctal invasive carcinoma, **M. Ciobanu et al**. realizes, using IHC, the research of orrelations between **the expression D2-40 LMVD, VEGF-C/ VEGFR-3** and ifferent histological and molecular types of **ductal invasive carcinoma**

esults: highlight the significant difference regarding the distribution D2-40 MVD, expressing higher positive values at the peripheries of tumors

lus, without taking into consideration the histological or molecular type, LMVD aried in the same direction both for VEGF-C and for VEGFR-3, the highest values eing registered in the positive peritumoral areas.

east cancer:

VEGF-C : an important role in the tumoral progression both through ymphangiogenesis and tumoral proliferation and also directly or hrough autocrine mechanisms directed over the cancer cells

- In breast tumors high levels of the expression VEGF-C were reported, with a strong correlation in the lymphatic vascular invasion, lymph nodes metastases and a poor prognosis.

VEGFR-3: expressed both in the lymphatic endothelium, as in the blood vessels of breast tumors, but its correlation with the lymph nodes status is controversial

There are studies showing the presence of the VEGFR-3 expression n lymphatic endothelium, especially in the periphery of the tumor, his being observed also at the level of small blood vessels

east cancer:

lalignant conversion takes place as the accumulation of mutations in the genes responsible r regulating the cellular division, apoptosis, invasiveness and metastasis

igh risk of breast cancer: BRCA1, BRCA2, TP53, PTEN, STK11 and CDH1

enes with reduced penetrance, which offer a high risk of breast cancer: ESR1, CASP8, GRF2, TOX3,MAP3K1, LSP1, 8q24.

he **polymorphism of ESR1 gene** and its correlation with the risk of breast cancer was udied by **A. Anghel et al.** which correlate the allele susceptibility with other clinic-athological parameters

Results: 6 SNPs were tested in the ESR1 gene, being obtained 4 specific phenotypes, which were correlated with significant clinic-pathological characteristics

lost studied: BRCA1 and BRCA2 genes

lutations in these genes=› lead to approx. 2% of the breast cancer cases

Burcoș et al. propose the evaluation of 9 mutations in gene BRCA1 and 2 on patients agnosed with breast cancer, in Bucharest.

Results: 114 cases were evaluated (100 females with sporadic breast cancer, 14 under family disease) and 7 mutations BRCA1 (185delAG, 5382insC, 943ins10, E1250X, 1294del40, E1373X, R1443X) si 2 in BRCA2 (IVS16-2A4G and 6174delT) were tested using PCR.

The results indicate **BRCA1 5382insC** as being the most frequent mutation revealed at women with breast cancer from the Romanian population

east cancer:

The superfamily GST: protective role against the alteration of DNA by exogenous and ndogenous oxidative agents

D.N. Chirilă et al. chose to evaluate the role of polymorphism of GST genes (GSTM1, GSTT1 and GSTP1), using PCR, in the development of breast cancer, or other cancers

- The results highlight **GSTM1 null genotype** as a **risk factor** in the development of synchroneous breast cancer and breast cancers associated at least with another extra-mammary tumor

ADAMs: includes over 30 proteins belonging to the family metzicin matrix-zinc depending f proteases, being transmembraneous proteins, multidomain, which secrete proteins with ole in adhesion, merger and cellular signaling

D. Narița et al. evaluate the role of the **gene ADAM 12** in breast cancer; the study proves tself remarkable through the way of obtaining the desired test, laser-capture microdissection, valuation through PCR of the the two kind ADAM 12-S –splicing variant, and L membrane-ound long variant

Results: a proof of involvement of ADAMs in the tumorigenesis and progression of breast ancer, leaving the challenge to new therapeutic targets.

east cancer:

yk (spleen tyrosine kinase): known to have several functions both in the immunity and orphology of epithelial cells

- In the normal breast tissue and in the epithelial tumor cells as well, Syk is responsible in preventing tumor growth, its loss of expression being associated with invasiveness and metastasis.
- The association between loss of expression for allelic Syk and tumor invasiveness, using FISH-fluorescent in sytu hybridization, was assessed.
- Results: loss of expression for alelic Syk, for both ductal breast carcinoma in situ and invasive breast carcinoma.

latrix-metalloproteinase: important in angiogenesis, apoptosis, "escape" mechanisms, anti-moral immunity mechanisms.

ffect on the DNA synthesis and methylation disruption of DNA caused by folic acid deficiency, ay increase the risk of cancer. Epidemiological studies proved the implication of a reduced nsumption of folate in the risk of developing breast and colorectal cancer, especially among cohol consumers.

common polymorphism is **A66G MTRR**, determined by the substitution of methionine with oleucine in position 22 (M22I).

Burcoș et al. propose the evaluatioan of possible associations between A66G MTRR s1801394) and the susceptibility to colorectal or breast cancer of Romanian patients.

he results of the study refute a significant statistical correlation between A66G MTRR s1801394) and the risk of developing a colorectal or breast cancer.

Colorectal cancer

lorectal cancer

- Third cause of death worldwide, after prostate and breast cancer
- Object of many studies, evaluating its molecular mechanisms
- Hope for new diagnosis and prognosis markers for colorectal cancer

lorectal cancer:

Carcinogenesis = 3 steps

- Transition from the normal colonic epithelium
- Pre-malignant adenoma stage
- Malignant stage

Genetic basis: a logical sequence of several steps, shaped by genetic mutations

lorectal cancer:

Great heterogeneity

Sporadic colorectal carcinomas:

- 85% display chromosomal instability (CIN) = › aneuploid and polyploid karyotypes
- 15% proved microsatellite instability (MSI)

orectal cancer:

MP-1, MMP-3, MMP-7, DEFA-1, DEFA-5, DEFA-6, IL-8, CXCL-1, SPP-1, CTHRC-1 gene pression involved in the onset and tumor progression?

Năstase et.al aim to assess the expression, using PCR and immunohistochemistry

esults:

- High levels of the **defensin 5 (DEFA-5)** and **defensin 6 (DEFA-6)** mARN in adenomas, were revealed, compared to the tumoral tissue
- **MMP-7**, whose levels are higher than those of MMP-1 and MMP-3, has similar expression in both adenomas, and tumors
- **MMP-7**: role in tumorigenesis and tumor progression
- **MMP-7**: correlation with hepatic metastasis
- **SPP1 (osteopontin):** higher in tumoral tissue, compared to adenomas
- **IL-8**: significantly higher levels, strongly expressed in adenocarcinomas
- **CTHRC1**: tumoral invasion and metastasis
- **CXCL1**: similar results for both adenomas and adenocarcinomas

lorectal cancer:

Iatrix-metalloproteinases (MMP):

- Overexpressed in many malignant tumors
- Directly linked to aggressiveness, tumor stage and prognosis

urlin et al: assess the clinicopathological and prognostic significance of the genetic pattern of netalloproteinases and their tissue inhibitors

- Results: they showed the need of an individual study for each MMP, useful in an easier correlation between each of them and cancer pathogenesis
- A meta-analysis would be necessary

Osteopontin (SPP1) : frequently being in co-localization with the MMPs, is a lycoprotein involved in bones development or in immune response

- Role in cancer, too, being associated with tumorigenesis, tumor invasion and metastasis
- Osteopontin gene =a transcriptional target of the aberrant Wnt signaling
- Its expression = a predictive survival factor in colorectal carcinoma

lorectal cancer:

L-8 : multifunctional cytokine, member of the CXC chemokines' superfamily

- Its receptors CXCR1 and CXCR2= present in both normal and tumoral tissue
- Key function in angiogenesis, tumoral growth, tumoral progression and metastasis, by inducing the chemotaxism

Overexpression= associated with a poor prognosis

I. Bălășoiu et al assess serum levels and IL-8 expression, in different stages of colorectal cancer

- Results: IL-8 levels were higher in the supernatant compared to those in the serum, proving that IL-8 is not produced only by the tumoral cells, but also by the healthy ones (endothelial cells, macrophages, neutrophils)
- Marker of poor prognosis

XCL1 (ligand-1 chemokine): a cytokine initially described in malignant melanoma, that seems to be volved in tumorigenesis, angiogenesis and metastasis of the colorectal cancer, too

THRC-1 (collagen triple helix repeated containing 1): a highly conserved in evolution glycosylated rotein

- Described as a TNF-beta inhibitor
- Produced, intermittently, after arterial injuries, in smooth muscles cells, fibroblasts, during neointima's development
- Considered to be involved in the tumoral invasion, as well.

lorectal cancer:

EA: one of the firsts studied biomarkers

- Seems to be correlated to a high risk of recurrence and poor survival
- Low molecular serum glycoprotein
- Role in facilitating metastasis to lungs and liver
- Important in postoperative follow-up of the colorectal cancer
- High levels= micro -metastasis

. Strâmbu et al claim, in their study, CEA's importance, alongside with **CA 19-9** and **A72-4**

- Evaluated during 3 years, on 324 postoperative patients
- Results: a relationship between advanced Dukes stage and high levels of the assessed markers

i-67: proliferative marker

- **Bolocan et al.** assess its expression
- Results: a correlation between ki-67 and the degree of transmural tumoral penetration
- Prognostic role, useful as a response parameter to radiotherapy in colorectal carcinomas

lorectal cancer:

irst steps in colorectal carcinogenesis= high cellular proliferation

CNA (proliferating cell nuclear antigen): a polypeptide

- Synthesized and expressed only by cells in the proliferation phase
- Seems to play an important role in DNA replication

GFR (epidermal growth factor receptor) mechanism: involved in the development and tumoral growth

- A proper understanding of the EGFR signaling cascade and KRAS mutations => therapies for advanced colorectal carcinoma

ncogene Ras family =3 members: **K-ras, H-ras si N-ras**

- All of them involved in human cancer development

-ras gene mutations: found in more than 50% of adenomas and colorectal carcinomas

M. Dobre et al assess K-ras expression

- Results: Positive results for K-ras mutations turn out in 47,4% of the evaluated cases, in codon 2
- The authors recommend PCR-RFLP for assessing high tumoral specimens, with rich cellularity , pyrosequencing fitting the small ones best

urzu et al claim the relationship between keratin 7 and K-ras, in distal colon localized cancers; keratin 7 ay signify, in this case, wild type K-ras carcinomas, responding well to Cetuximab or Panitumumab erapy- anti-EGFR antibodies

lorectal cancer:

errated pathway adenocarcinomas: two forms

- The traditional one, polypoid
- The non-polypoid form

Gurzu et all: attempt a molecular, immunohistochemical, linicopathological analysis of the colorectal carcinomas

- To identify molecular and immunohistochemical criteria => an easier recognition of the serrated pathway adenocarcinomas
- Assess the **CK7, CK20** expression- in membrane and cytoplasm, and p53 nuclear expression-using immunohistrochemistry-, and gene mutations **BRAF (V600E)** and **K-ras (codon 12,13)**, using PCR
- Results: sporadic right colon carcinomas- MSI-BRAF => good prognosis and low risk for distant metastasis
    - MSI-BRAF wt or MSI-BRAF-mut => poor prognosis

lorectal cancer:

Gene mutations: also assessed in a cohort study, on Greek and Romanian patients

- Results: new protocol established- HRM detecting method, for **K-ras** (exon 2,3), **NRAS** (exon 2,3,4), **BRAF** (exon 15)

-53: tumor suppressor gene

- Deletions and mutations identified in more than 85% of colorectal carcinomas
- Occurrence frequently seen in the transition phase, from adenoma to adenocarcinoma

C. Ghiță et al =›p-53 gene mutation= seen in 19 from the 22 evaluated cases

- More frequently = in distal colon cancers and rectal cancer
- Correlated to the age of the patients, and with a poor prognosis

Bcl-2: oncoprotein, role in inhibiting apoptosis

- Bcl-2 gene overexpression being seen in a several malignant tumors
- Bcl-2 expression = assessed by **C. Ghiță et al**
- Results: an overexpression, rarely seen in colorectal cancer, especially in incipient phases, correlated to a good prognosis of these patients.

lorectal cancer:

RAF mutations:

Almost 10% of the colorectal carcinomas seem to harbor this mutations

BRAF V600E= the most frequent one

Prognostic role= not completely clarified

Gurzu et al. : identifying MSI-BRAF mutant right colon carcinoma, with a MLH-1 expression and Keratin-7 positive expression => diagnostic criteria for a good response at Oxaliplatin therapy

- Almost 17% of the colorectal carcinomas express **CK7**
- Almost 18%, don't have a **CK20** expression
  - CK20= long enough associated with colon metastasis and their differentiation from other types of metastasis
  - **Gurzu et al.** assess these markers' expressions= › a more precise quantification of their importance
    - Correlation with microsattelite status and BRAF mutations
    - Results: CK7-/CK20 + phenotype= characteristic to colorectal carcinomas
    - CK positive expression in lymph nodes metastasis, self-dependent of other factors
    - CK20 expression =negative for BRAF mutant carcinomas

lorectal cancer:

enomic instability: 2 features =› different genetic variants of tumorigenesis

- One form- in nucleotides, resulting in frequent deletions and insertions at this level=**microsattelite instability** (MSI)

- Characterized by the accumulation –during DNA replication- of several successive mutations, errors normally corrected by mismatch repair genes -MMR (1,2,3,4,5,6), due to the inactivation of 4 genes: MSH2, MLH1, MSH6, PMS2

- **Ioana et al** : assess the pattern of the nine **MMR genes** (ANKRD17, MLH1, MLH3, MSH2, MSH3, MSH4, MSH5, MSH6) in normal tissue, in pre-malignant lesions (adenomatous polyp) and malignant sporadic colorectal carcinomas.

- Assessment done by PCR

- Results: MMR overexpression is associated with malignant proliferation and a good prognosis

urzu et al: support the prognostic role of **microsattelite instability**

- Results: a worse prognosis in MSI-H compared to MSI-L/MSS

.Bolocan et al: relationship between polyp-colorectal cancers= ›the importance of a well-one screening

- Genetic testing, accessible to all patients

- APC, DCC, p53 testing = only in properly-equipped laboratories.

lorectal cancer:

Iolecular instability cause:

- 10% of sporadic colorectal carcinomas
- 90% of hereditary non-polypoid (Lynch syndrome)

. **Mureșan et al.** use the **microsattelite instability** and **loss of expression** for one of the **genes hMLH1 or hMSH2**, in rder to identify potential patients wih Lynch syndrome

- Assessment: using immunohistochemistry
- Results: 13 patients with loss of expression for one of the targeted genes were identified
- 7 patients with a potential Lynch syndrome
- 110 patients with colorectal carcinomas evaluated

TEN= tumor supressor gene

- Its mutational inactivation leads to carcinogenesis and tumoral proliferation
- **Ghiță et al**. assess its immunohistochemical expression
- Results: negative in a sole case, and weak-expressed in 2 others, out of all 22 tested
- PTEN mutations= certified in distant colon, usually associated with advanced stages of disease

Iaspin serine-protease (Maspin B5), coded by Maspin gene: antiproliferative activity, antiangiogenic and roapoptotic effects

- **Gurzu et al** aim to assess this gene, create a new quantification system for it and correlate it with other histopathologic and molecular parameters
- Results: a positive expression, especially in the cytoplasm, usually correlated with microsattelite instability.
- Literature data correlates the nuclear positive expression with tumor dimension, poor survival and local

olorectal cancer:

The GST superfamily : protective role against DNA alteration by exogenous and endogenous oxidative agents

- **D. N. Chirilă** et al aim to evaluate the genetic polymorphism of three glutathione S-transferase (GSTM1, GSTT1, and GSTP1) in patients with multiples colorectal cancers or a colorectal cancer associated with another type of cancer
- **GSTM1 genotype:** A high risk for colorectal cancer, or for an association between colorectal cancer with another type of cancer

L.M. Procopciuc and **G. Osian** aim to evaluate GSTM1-null's role as a risk factor in colorectal cancer, starting from a correlation between **GSTM1genotype/NAT2 acetylator phenotype**, **smoking** and **high intake of red fried meat** among Romanians, women or men

- Results: smoking and high intake of red fried meat= risk factors for colorectal carcinoma
- **GSTM1-null/NAT2*6B rapid acetylator phenotype** in association with **smoking, without a high intake of red fried meat**, reveals a **risk for colorectal cancer**, compared to non-smokers, with the same diet

olorectal cancer:

Drug-resistant **cancer stem cells** (CSC)= leading cause of drug-resistance in colorectal cancer's treatment

- **CD133** and **CD166**= surface markers of cancer stem cells
- **Mărgăritescu et al**. assess the individual expression and the co-expression of these markers, in order to evaluate their prognostic role
- Assessment: by using immunohistochemistry fluorescence
- Results: a relationship between CD133 and CD166' co-localization and early colonic tumorigenesis was seen
- CD133 and CD166 could be use to classify colorectal cancers and as prognostic markers, as well, especially in early stages disease and differentiated tumors

olorectal cancer:

Molecules with a controversial role in colorectal cancer

- **TIMP-1**: described with antitumor properties, despite of other studies, according to which it has a role in tumorigenesis
- **I-CAM1** seems to have a role in tumor pathology as well, not only in cell adhesion and migration
- **E-Cadherin** (CDH1), associated with several genetic changes
- assessed by **Ionescu et al**
  - Results: a strong expression of I-CAM and TIMP-1 in tumors

The renin-angiotensin-aldosteron system (RAAS) : involved in several processes like cell proliferation, apoptosis, immune response, and in several cancers, as well

- **The angiotensin I-converting enzyme** (ACE): a key role in RAAS, inhibiting angiotensin I transformation in angiotensin II, strongly vasoconstrictive
- **M. Toma et al**. aim to assess possible associations between ACE gene I/D polymorphism and colorectal cancer in Romanian patients
- Results: no significant association was seen between those entities

L.M. Procopciuc and **G. Osian** aim to analyze **gene polymorphisms in XPD** (Lys751Gln) and XRCC1 (Arg399Gln), among patients with colorectal cancer, assessing the risk for colorectal cancer given by these SNPs, and their correlation to several other tumor pathology characteristics

- Results: an association, both in women and men, between the **risk for colorectal cancer** and **Arg399Gln polymorphism**

lorectal cancers:

N-acetyltransferase 2= an N-acetyltransferase isoenzyme, usually found in liver and intestinal mucosa

- Role in catalyzing N-acetylation and O-acetylation of different pro-carcinogenic and heterocyclic amines
- The prognostic role of NAT2 mutations =studied by **G. Osian** and **L. Procopciuc** considering that individuals with rapid acetylator phenotype are more exposed to high amounts of environmental carcinogenic, with a higher risk for colorectal cancer
- Results: a possible influence towards disease evolution of NAT2 mutational status

lorectal cancer:

Genome-wide association studies (GWAS)= new opportunities in identifying other common enetic variants involved in colorectal etiology

- Usually, case-control studies

N. Mateș et al, collaborating with deCODE Genetics, Iceland aim to test some previously alidated **SNPs,** evaluating their distribution in Romanians

- 8 SNPs were evaluated, of which 6 were selected based on previous research, the other 2 being validated by Cogent study
- Results: a link between **rs6983267, rs4939827, rs3802842, rs4444235, rs10795668** and colorectal cancer risk was observed

utophagy= intracellular catabolic process in which intracytoplasmic particles are being equestered and 'given' to lysosomes

- Mutations in autophagic genes may lead to changes in gastric microflora, then to gastro-intestinal disease
- Mutations in this genes at a young age (<30 years), increase the risk for colorectal cancer in these patiens
- Loss of expression for autophagic protein ATG16L1 facilitates endotoxin-induced cytokine's production, acting as an inflammation modulator, with an influence towards tumor cell survival in different types of cancer
- **Nicoli et al** assess ATG16L1 +898A>G polymorphism' role in the onset and progression of colorectal cancer,
- Results: a risk for the GG genotype, especially in men, was outlined

lorectal cancer:

Tumor-infiltrating lymphocytes (TIL): a recognized prognostic factor ince 1987

- Seems to be a marker of a good prognosis in colorectal cancer
- It was claimed that lymphocytes involved in anti-tumor defense have a certain role in preventing tumor cell progression
- Its subtypes: CD4+T helper cells, CD8+T cytotoxic cells, and regulatory T cells (Tregs): role the in oncologic follow–up

Circulating tumor cells (CTC) and **angiogenesis**: new prognostic factor

- Assessed by immunohistochemical analysis of neoformation vessels
- **VEGF**- a well known angiogenic marker, its high levels being associated with a poor prognosis

lorectal cancer:

Gastro-intestinal stromal tumours (GISTs) : rare gastro-intestinal mesenchymal tumors

- A high malignant potential and an unpredictable behavior
- Their association to other tumours is rare, only few cases being quoted in literature
- **C. Nemeș et al** present such a case of association between a GIST and an adenocarcinoma, in a 61-years old man
- An explanation for the simultaneous occurrence of the two tumors: mettalothionein, a mettaloproteinase increasing the affinity for heavy metal ions

S. Ștefaniță et al aim to evaluate the inflammatory cells from the **peritumoral stroma**, and their role in inflammatory peritumoral reaction

- Results: The study, evaluating 23 adenocarcinomas, highlights a variable chronic inflammatory peritumoral infiltrate
- Among these cells, mastocytes and macrophages were best represented, the B lymphocytes being least represented
- Chronic inflammation=a process shaped by lymphocytes, mastocytes, plasmocytes and macrophages at the site of inflammation, communicating through chemokines , until complete restoration of tissue integrity is done
- The inflammatory process may inhibit tumour development, or, on the contrary, facilitate it, trough different signaling pathways

Prostate cancer

rostate cancer

First place worldwide -malignant tumors in men

Second cause of death due to malignancy in Europe

Prostate cancer: needs more diagnostic biomarkers capturing the disease in early stages=› improving the prognosis, and the quality of life

ostate cancer:

SA-prostate specific antigen: the most used biomarker

- Not very specific and sensible=› its role as a screening tool was doubted several times
- Increasing need of a ‘model’, able to define normal value of PSA
- Sequence variants must be considered in such a definition, as gene control by cis and trans components has already been established
- 40-50% of the PSA levels variability among men= due to genetic inheritance

LK3 gene=involved in coding PSA

- a high risk variant for prostate cancer was found, **rs2735839-G**, near KLK3 gene

WAS: evaluates sequence variants responsible for the variability of PSA levels

- Results:6 loci correlated to PSA levels
- Missense mutation **rs17632542-T**, in KLK3 gene is associated to high levels of PSA

esearch strategies’ aim: to change the classical protocols

- Better prognosis

ne answer : **biologically adapted treatments**

ostate cancer:

Cancer stem cells (CSC): a cause of resistance to treatment

Beta 2 receptor expression, of the retinoic acid (RARβ2): has the behavior of a tumor uppressor gene

- Expression was seen in many malignant tumors, among which prostate cancer
- Hypermethylated expression: useful in differentiating benign lesions from the malignant ones
- **R. Dumache et al** aim to assess, using PCR, the hypermethylated expression of RARβ2, from the serum of both patients with prostatic cancer and healthy individuals
- Results: an early stage, non-invasive diagnostic marker

L. Bălăcescu et al. evaluate up and down gene regulation mechanisms involved in prostate cancer progression

- Results: using Tissue Microarray Technique, 1119 genes, with different expressions, were identified, of which 378 up-regulated and 741 down-regulated
- An important genetic network was seen around TERT, BCL2, and SMAD3 genes
- An overexpression of TERT, which is known as a potent antiapoptotic modulator was seen
- New information on different genes involvement in the molecular network of prostate cancer, compared to normal tissue

ostate cancer:

Mastocytes: highly studied, because of their particular response to different stimuli, ich in mediators intracellular containing, and different phenotype

Correlatation with angiogenesis, tissue remodelling, or stromal immune modulation

Role in cancer= still controversial

T. Globa et al. aim to assess their role in benign and malignant lesions of the prostate, creating them an immune phenotype at this level

Results: a high amount of mastocytes were identified in normal prostate, using 3 markers: tryptase, chymase and CD117

Using of the same markers, different immune phenotypes of mastocytes, in benign and malignant prostate tissue, were highlighted

Heterogeneous expression of mastocytes in prostate benign and malignant lesions

Lung cancer

ung cancer:

Requests permanently innovative research in order to understand its developmental mechanisms, differentiation and tumor progression

Classification: squamous cell carcinoma (SC), adenocarcinoma (ADK), large-cell lung cancer (LCLC) and small-cell lung cancer (SCLC)

Daily practice classification: small-cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC)

ng cancer:

nteractions between **tumor** and **peritumoral stroma**= of great importance

nvolvement of stroma and extracellular matrix in tumor progression =› new potential herapeutic target

umor-infiltrating lymphocytes (TIL)

- Recent data regarding regulatory **T cells (Tregs)=›** new perspectives on TIL's pro and anti tumor implications.
- Tregs: CD4+CD25+ immune phenotype
- A potent immune modulator capacity, especially in keeping the immune tolerance, by controlling response and immune regulation of both CD4+ and CD8+
- **Foxp3 expression**: recently added to Tregs immune profile
- **Th-17**, is responsible of synthesizing IL17=› involved in inflammation and autoimmunity

. Vasilescu et al aim to analyse Foxp3 expression, correlating it with IL17, in Tregs and umor cells, from adenocarcinomas, using immunohistochemistry

esults: 59 evaluated cases= › TIL= poor prognosis

oxp3 –tumor progression- a new therapeutic target

ng cancer:

Most used markers in lung tumors: **TTF-1, CK7, CK20, Anti-CA 15-3 antibody**

- Epithelial markers: **CK AE1/AE3, CK5/6, BER-EP4, KL-1, ACE, PK EMA, CD56**
- Neuroendocrine markers: **Chromogranin –Chromo, Synaptophysin – Syn, Neuron Specific Enolase NSE**

arely used markers: **Anti-thyroglobulin antibody, BHCG, PLAP, PSA, Anti-yrocalcitonin antibody, AFP, Panleuco-1, CD3, CD20, CD30, CD45, CD99, D117, CD141, Vimentin, Desmin, Actin, cadherin, Calretinin**

. Demetrian et al assess their expression

- Attempt in creating a profile for lung carcinoma
- Results: Tumors showed a great morphologic variety
- Imunohistochemistry= priceless tool in lung tumors diagnosis

ng cancers:

mmunohistochemistry= helpful in neoplastic pleural effusions diagnosis

leural effusion= a frequent diagnosis problem in Pneumology, because of the vast athology, benign and malignant, causing it

M.Dinu et al assess **calretinin, HBME1, D2-40, Ber-EP4, CK5/6, CEA** and **TTF1expression** in 37 cases of pleural and lung tumors.

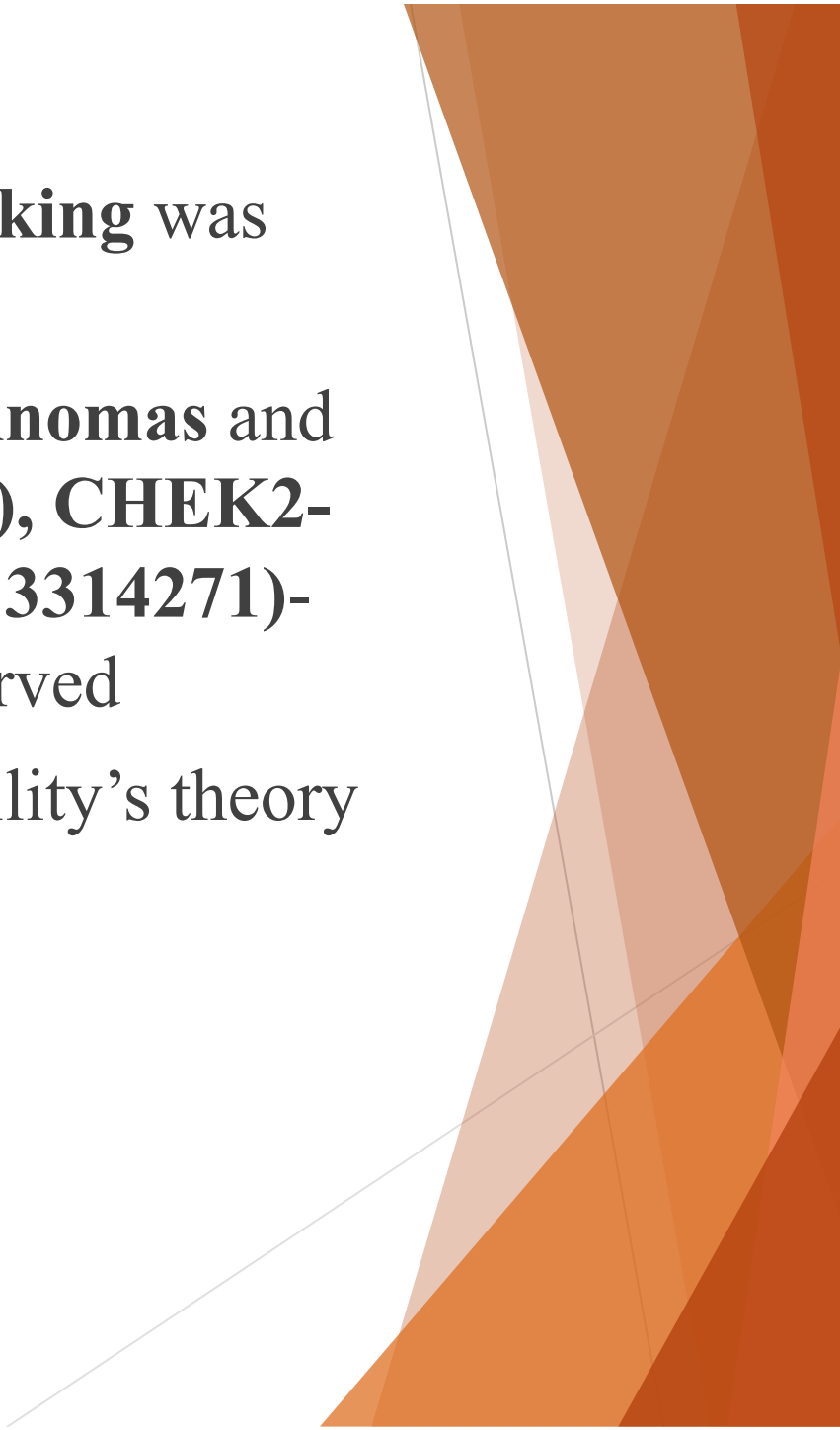
- Results: Out of 37 cases, 5 proved to be epithelial mesotheliomas, the others being lung tumors and pleural metastasis.
- Calretinin, D2-40 si CK5/6 = specificity for mesotheliomas
- Ber-EP4, CEA and TTF1=specificity for adenocarcinomas
- Ber-EP4, CK5/6 and CEA = specificity for squamous cell carcinomas
- Helpful for lung tumors and mesothelioma's diagnosis

Genetic instability: studied in lung cancer, as well

GWAS were conducted in order to identify **new lung cancer susceptibility loci**

ng cancer:

- A link between identified **phenotypes** and **smoking** was evaluated
- New correlations between **squamous cell carcinomas** and rare variants of **BRCA2-K3326X (rs11571833), CHEK2-I157T (rs17879961)** and with **3q28 (TP63, rs13314271)**- previously identified only in Asians, were observed
- New variants= new proofs in genetic susceptibility's theory of lung cancer and its biologic basis.



oncluding, it can easily be seen that Romanians
esearch in the last 5 years led to valuable novelties in
ncer genetics, with a tendency to breast cancer and
lorectal cancer.