A Clinical Use of DNA Methylation Analysis: Diagnosis of Cancer of Unknown Primary Using EPICUP

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CpG-rich genes

Normal Cells

Cancer Cells

RB, P16
MGMT
GSTP1
BRCA1
MLH1

Gain

HOXA5
HOXA9
MASPIN
MCJ

Gain

Loss

MAGE
CAGE
BAGE
LAGE

CpG-poor genes

Berdasco and Esteller, Developmental Cell, 2010
Cancer of Unknown Primary

Fernandez et al., Genome Research, 2012
Malignant neoplasm, histologically proven, whose primary origin is not known after performing standard and complementary clinical approach:

- Physical examination
- Laboratory and radiographic studies
- Full histopathology

Facts

- Cancer is the leading cause of death worldwide and its estimate progression for 2030 is 13.1M.
- 3 to 5% of total cancer are Cancers of Unknown Primary (CUP).\(^1\)
- Definition

Cancer of Unknown Origin

- CUP incidence is strongly age related.
  - 55% of cases were diagnosed in those aged 75 and over
  - 77% of cases were diagnosed in those aged 65 and over
Cancer of Unknown Primary

Standard diagnostic approach

Panel 1 - Investigations that should be done before diagnosis of CUP in patients with suspected CUP

Clinico-pathological data
- Histologically confirmed metastatic cancer
- Detailed medical history
- Complete physical (including pelvic and rectal examination)
- Histopathological review with specific immunohistochemical study

Laboratory test data for all patients
- Full blood count
- Biochemistry
- Urinalysis
- Testing for occult blood in stools
- Chest radiography
- CT scan of thorax, abdomen, and pelvis

Laboratory test data for selected patients only
- Mammography (for all women)
- Breast MRI
- Transabdominal ultrasound
- PET or CT scan
- Concentrations of serum or heparinase and/or human chorionic gonadotropin
- Concentrations of serum prostate-specific antigen (for all men)
- Concentrations of serum cancer antigen 125 and carcinoembryonic antigen
- Lactate dehydrogenase

Epigenetic profiling to classify cancer of unknown primary: a multicenter retrospective analysis.
Validation & Discovery Cohorts (n=10,481)

Specificity
- PEBC: 2,640
- TCGA: 7,841
- Prymary Tumor: 9,858
- Metastasis: 619

Sensitivity
- 99.6%
- 97.7%
EpiCup Prediction in CUPs Cohort

Compatible with Histology

96.1%
Efficacy of Epigenetic Profiling to Classify Cancer of Unknown Primary

Moran et al., The Lancet Oncology 2016
Site-specific therapy for CUPs confers improved survival: Prospective studies

Hainsworth et al., J Clin Oncol 2013
Yoon et al., Annals of Oncology 2016
Varadhachary et al., Clin Cancer Res 2011
A  Overall Survival, according to therapy

Specific therapy  (n = 31; 13.6 m)

Log-rank; p-value = 0.003

HR (95% CI) = 3.24 (1.42 – 7.38)

Empiric therapy  (n = 61; 6.0 m)

Months

Since Diagnosis

Overall Survival

Patients at risk:

Therapy

Specific

31

31

31

29

25

24

Empiric

61

59

46

38

34

30

C   IHC and molecular characterization of CUP case predicted as NSCLC by EPICUP

H&E

CK7

CK20

TTF-1

TTF-1

C-MET amplification

EGFR mutation in exon 18

B   Multivariate Analysis

Patients features  (N= 92)

Gender

Age

Diagnostic method

Histology at diagnosis

Histology at biopsy site

Multiple metastasis sites

Specific therapy

Hazard Ratio for OS (95% CI)

G791X

Control

H&E

CK7

Specific therapy

0.99 (0.87 – 1.10); p=0.976

0.96 (0.69 – 1.33); p=0.809

2.03 (0.94 – 4.09); p=0.117

0.30 (0.23 – 0.39); p=0.976

1.09 (0.55 – 2.18); p=0.799

1.15 (0.72 – 1.85); p=0.595

0.96 (0.65 – 1.30); p=0.809

0.84 (0.65 – 1.09); p=0.907

0.64 (0.32 – 1.28); p=0.209

0.82 (0.72 – 1.00); p=0.796

Risk

Benefit

HR (95% CI)

95% of Confidence Interval (95% CI)

Hazard Ratio (HR)

Diagnostic method

Biopsy site

Multiple metastasis sites

Specific therapy

1.15 (0.72 – 1.85); p=0.595

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D   IHC and molecular characterization of CUP case predicted as NSCLC by EPICUP

Efficacy of Epigenetic Profiling to Classify Cancer of Unknown Primary

Moran  et al., The Lancet Oncology 2016
Case 1
Case 1

Gastric Cancer
Case 1

Gastric Cancer

FISH HER2
Case 1

Gastric Cancer

FISH HER2

TRASTUZUMAB
Case 3
Case 3

EXOME SEQUENCING
Case 3

EXOME SEQUENCING

BRAF MUTATION
Case 3

EXOME SEQUENCING

BRAF MUTATION

BRAF INHIBITOR?
Case 3

EXOME SEQUENCING

BRAF MUTATION

BRAF INHIBITOR?
Case 3

EXOME SEQUENCING

BRAF MUTATION

BRAF INHIBITOR?

COLON CANCER
Case 4
Case 4

epicup
Case 4

Breast Cancer
Case 4

Breast Cancer
Case 4

Breast Cancer

Breast Cancer Family History
Case 4

Breast Cancer

Family History

BRCA1 Mutations
A DNA Methylation Fingerprint
Table 7: Hypomethylated gene predictor drug sensitivity

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene function</th>
<th>Therapeutic consequences</th>
<th>Tumour type application</th>
<th>Example role</th>
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<tbody>
<tr>
<td>ABCG2</td>
<td>Protein transport</td>
<td>Sensitivity to doxorubicin</td>
<td>Breast</td>
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<td>ARF1</td>
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<td>Resistance to anthracyclines</td>
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<td>BRCAn1</td>
<td>DNA damage response</td>
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<td>CDH11</td>
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<td>Sensitivity to paclitaxel and paclitaxel</td>
<td>Breast, colon, stomach</td>
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<td>Sensitivity to cisplatin</td>
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Aim: Predict tumor of origin for Cancer of Unknown Primary patients