Cancer Cell DNA Replication: Mining for Biomarker and Therapeutic Discovery

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A variety of factors promote the transformation of the normal human cell to a tumor cell.

The malignant cell hosts a wide number of changes in phenotypic properties.

The cancer cell accumulates genetic damage.

The accumulation of genetic damage correlates with cancer progression.
Current Concepts in Cancer

There is a Need for New Biomarkers (or molecular detectors) for Cancer

- Early detection of disease is a key
- The weaknesses of current crop of biomarkers include:
  - Lack of specificity
  - Appear later in the disease
  - Reliability issues
  - Can’t be used as a screening tool
The Potential Application of Tumor Markers

- Screening healthy subjects or a high risk group for the presence of tumors
- Identifying a specific type of cancer
- Determining the prognosis of a cancer patient
- Monitoring the effectiveness of therapy in a patient in remission or while undergoing therapy
Current Concepts in Cancer

There is a Critical Need For New Cancer Therapeutic Targets

- Specific intervention is key
- The weaknesses of many of the current crop of therapeutics
  - Lack of specificity
  - Adaptability of cancer cells to develop resistance to therapy
  - Inability to customize treatment
Experimental Approaches to Discover Cancer Biomarkers

- **Proteomics** is a field of study exploring the structural and function relationship of all the proteins within the cell.

- **Genomics** is a field of study exploring the genetic make-up and expression of cells.

- **Functional Genomics/Proteomics** refers to the complex interaction between the two disciplines where the clustered expression of specific genes leads to the expression of “complex machinery” within cells that mediate specific cellular processes.
Human Cell DNA Synthesome

- Pol α
- RFC
- Primase
- RPA
- PARP
- Pol δ
- Pol ε
- Ligase
- PCNA
- Topo II
- Topo I
- DMT
- Direction of replication fork movement
Early Elongation Phase of DNA Replication
Late Elongation Phase of DNA Replication
Alteration of the DNA Synthesosome in Malignancy
Fidelity of Breast Cell DNA Synthesome Replication
DNA Replication Fidelity of the Malignant and Non-Malignant Human Breast Synthesome
PCNA is an integral component of the human cell DNA replication machinery.

Diagram showing various proteins involved in DNA replication, including PCNA, Pol α, Pol δ, RFC, Primase, RPA, Topo I, Topo II, Ligase, PARP, and Pol ε.
PCNA — “The Ringmaster of the Genome”
2D Gel Electrophoresis

(1) IEF or IPG

(2) SDS-PAGE

M.W. high to low
PCNA Resolution Profiles in Non-Malignant and Malignant Breast Cells Grown in Cell Culture
Resolution Profile of PCNA derived from Human Breast Tumor
The Protein Resolution Profile of PCNA from Benign Breast Samples
Is the Altered form of PCNA a Function of Genetic Mutation?
The Cancer Associated Form of PCNA (caPCNA) is Present in Patient Serum

A Potential Biomarker for Cancer?
Development of the **First** Antibody for the Detection of the Cancer Associated Form of PCNA (caPCNA)
PCNA
The Antibody Detects caPCNA Specifically

37kDa — csPCNA  nmPCNA
24kDa —
37kDa —
24kDa —

PC10 Ab

csPCNAab
The Antibody Detects Breast Cancer
Cells Grown in Culture

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Antibody Detects Cancer in Tissue

Commercially Available Antibodies

- PC10
- C20
- 100-478

caPCNA Antibody
The Antibody Detects Cancer Cells Specifically in Tissue

- Normal
- Invasive
- Metastatic
The Antibody Aids in the Diagnosis of Difficult Conditions

- It stains cells expressing caPCNA
Human cells contain a multiprotein complex that carries out DNA replication, termed the synthesome. The synthesome is mutated in cancer cells. csPCNA is a component of the synthesome. It is a potential marker for breast cancer.
Potential Translational Implications

- Since caPCNA has been found in 100% of all malignant cells tested to date and in the serum of untreated cancer patients it may prove to be a powerful new epigenetic pan-maker for cancer.

- caPCNAab may be useful for the detection of early stage cancer.

- The antibody may better define patients truly at risk for cancer in some cases.

- caPCNAab could potentially be effective for evaluating cancer therapeutic response.

- The antibody may have utility for monitoring the disease-free status of cancer patients following therapy.

- caPCNAab could possibly better define surgical tumor margins.