Cancer, Markers, and Bioinformatics

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What is Cancer?

Cancer is a large group of diseases characterized by the uncontrolled growth and spread of abnormal cells.

Neoplasm: new growth of tissue that serves no physiological function.

Tumor : clumping of neoplasmic cells.

Malignant : cancerous.

Benign : noncancerous.

Biopsy : microscopic examination of cell development.

Metastasis : malignant tumors that are not enclosed in a protective capsule have the ability to spread to other organs.

Mutant cells : disruption of RNA and DNA within normal cells may produce cells that differ in form, quality and function from the normal cell

* Lung cancer is the most common cancer in men.

•Breast cancer is the most common cancer in women.

Classification of cancers: 1- Carcinomas. 2- Sarcomas. 3- Lymphomas. 4- Leukemias. 5- Adenomas

A- Division (Uncontrolled cell division):

1- Oncogenes. 2- Tumor suppressor genes – p53. 3- Suicide genes – apoptosis

4- DNA repair genes.

B- Growth (Formation of a lump (Tumor) or large numbers of abnormal white cells in the blood):

Tumor

1- Pressure on nerves. 2- Blocking organs. 3- Stopping normal function

4- Altering nerve signals. 5- Fungating.

C- Mutation (Changes to how the cell is viewed by the immune system) and Spread (The ability to move within the body and survive in another part) :

1- Invasion.

2- Angiogenesis.

Tumor

1- Benign Tumors

- Self-limited in their growth.
- Do not invade or metastasize (although some benign tumor types are capable of becoming malignant).

2- Malignant Neoplasm or Tumors (Cancer)

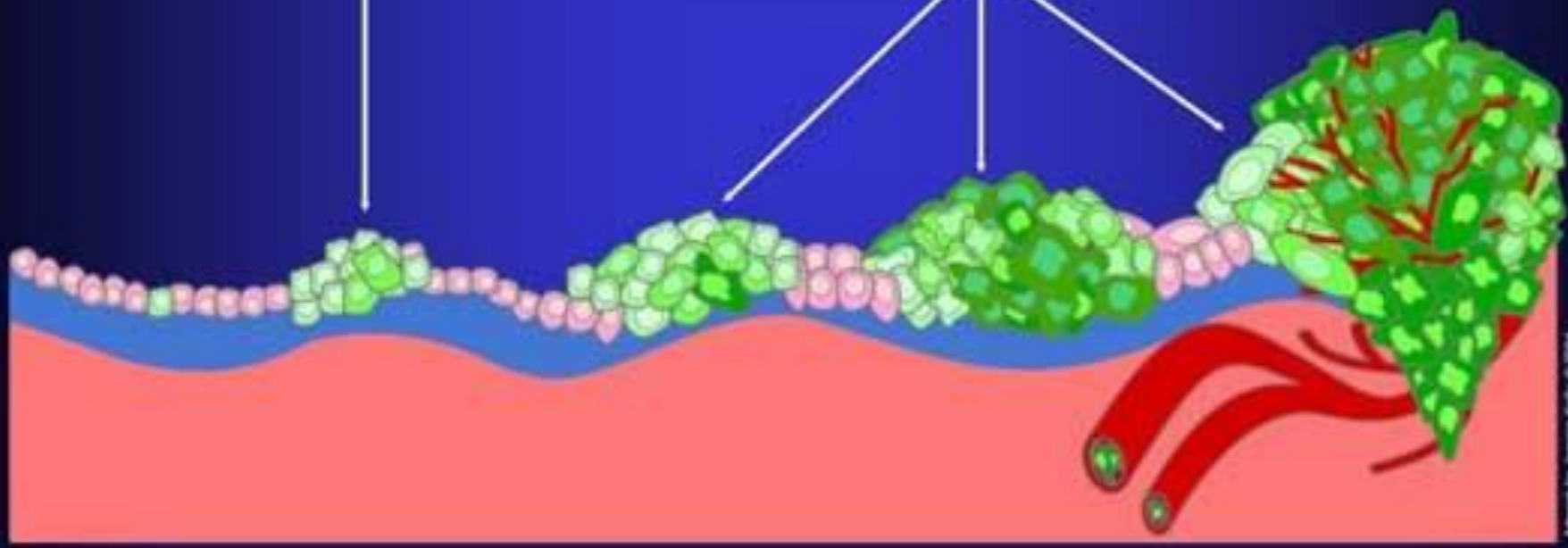
Group of cells display:

- Excessive growth and division without respect to normal limit,
- Invasive, invade and destroy adjacent tissues, and sometime,
- Distant metastasis spread to other locations in the body.

Malignant versus Benign Tumors

Benign (not cancer) tumor cells grow only locally and cannot spread by invasion or metastasis

Malignant (cancer) cells invade neighboring tissues, enter blood vessels, and metastasize to different sites

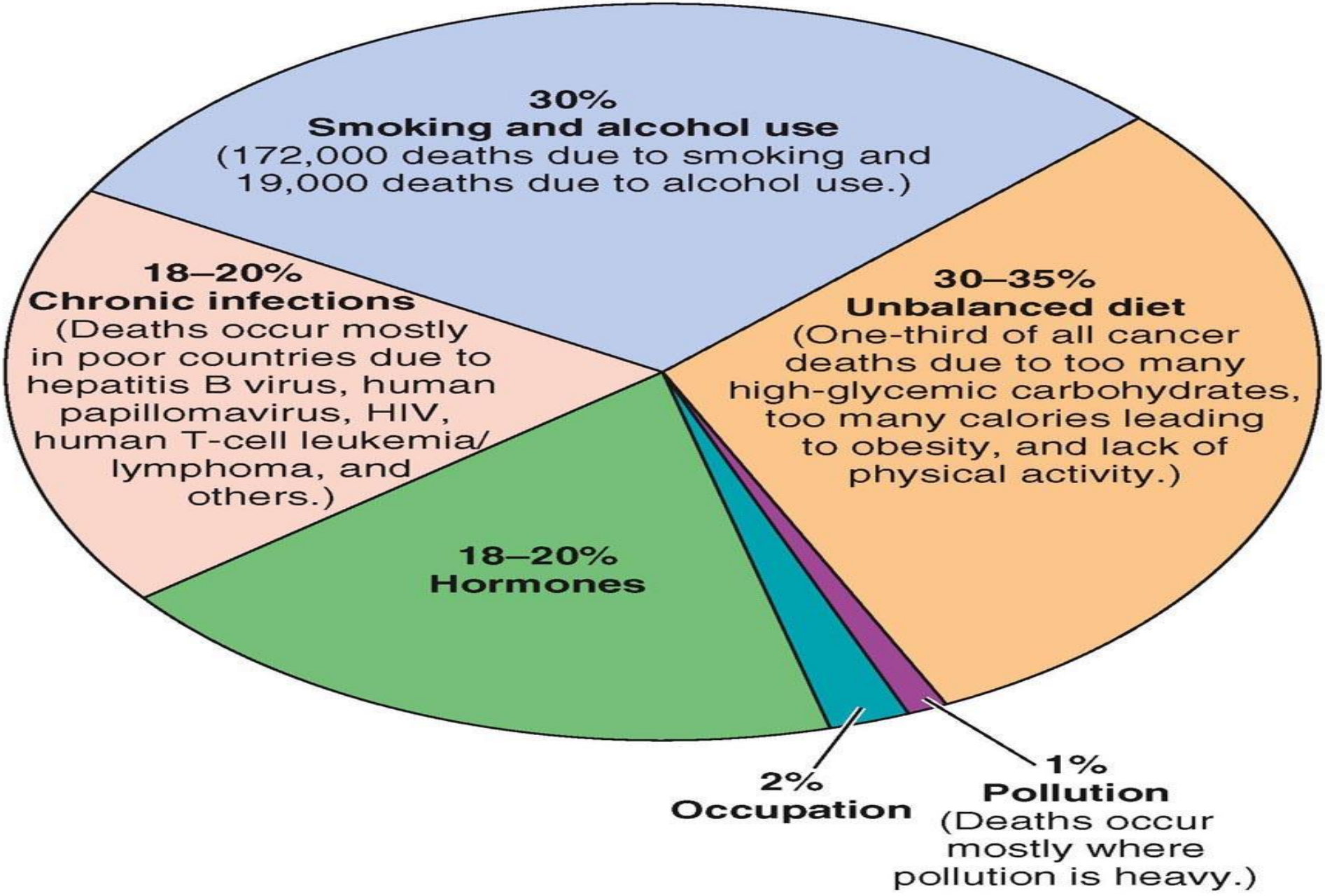


Time

What causes cancer?

- Cancer arises from the mutation of a normal gene.
- Mutated genes that cause cancer are called oncogenes.
- It is thought that several mutations need to occur to give rise to cancer
- Cells that are old or not functioning properly normally self destruct and are replaced by new cells.
- However, cancerous cells do not self destruct and continue to divide rapidly producing millions of new cancerous cells.
- A factor which brings about a mutation is called a mutagen.
- A mutagen is mutagenic.
- Any agent that causes cancer is called a carcinogen and is described as carcinogenic.
- So some mutagens are carcinogenic.
- Ionising radiation – X Rays, UV light
- Chemicals – tar from cigarettes
- Virus infection – papilloma virus can be responsible for cervical cancer.
- Hereditary predisposition – Some families are more susceptible to getting certain cancers.

Factors Believed to Contribute to Global Causes of Cancer



Detecting Cancer

1- The earlier the diagnosis the better the prospect for survival

2- Magnetic resonance imaging (MRI)

3- Computerized axial tomography scan (CAT scan)

4- Prostatic ultrasound

5- Regular self-exams, and check ups

New Hope In Cancer Treatments

1- Remove less surrounding tissue during surgery

2- Combine surgery with radiation or chemotherapy

3- Immunotherapy

4- Cancer-fighting vaccines

5- Gene therapy

6- Stem cell research

Markers

1- Biomarkers.

2- Molecular markers.

3- Cancer markers

Biomarkers

Biomarker is a substance used as an indicator of a biologic state

- Existence of living organisms or biological process.
- A particular disease state
- A fragment of DNA sequence

Location of the biomarker:

1- Location of a particular molecule can also be a marker

2- Cellular-subcellular locations

3- Tissue or organ locations

For example:

1- Galectin-3 : is a member of lectin family. It is wildly distributed In tissue of epithelial, fibroblast, and dendritic cells.

2- Blood Galectin-3: Predict the outcomes for patients with symptoms of heart failure

Detection of biomarker:

1- Diagnosis: Self properties, e.g enzymatic activities, Antibodies, IHC, ELISA

2- **Quantitative** : a link between quantity of the marker and disease

3- **Qualitative** : a link between exist of a marker and disease

Biomarker conditions for Screening:

1- The marker must be highly specific, minimize false positive and negative

2- The marker must be able to clearly reflect the early stage of disease

3- The marker must be easily detected without complicated medical procedures.

4- The disease markers released to serum and urine are good targets for application of early screening.

5- The method for screening should be cost effective.

Samples for biomarker detection:

1- Blood, urine, or other body fluids samples

2- Tissue samples

Molecular marker

- Molecular marker are based on naturally occurring polymorphism in DNA sequence(i.e. base pair deletion, substitution ,addition or patterns).
- Genetic markers are sequences of DNA which have been traced to specific locations on the chromosomes and associated with particular traits.
- It can be described as a variation that can be observed.
- A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like mini satellites.

Some commonly used types of genetic markers

- RFLP (or Restriction fragment length polymorphism)
- AFLP (or Amplified fragment length polymorphism)
- RAPD (or Random amplification of polymorphic DNA)
- VNTR (or Variable number tandem repeat)
- Micro satellite polymorphism, SSR (or Simple sequence repeat)
- SNP (or Single nucleotide polymorphism)
- STR (or Short tandem repeat)
- SFP (or Single feature polymorphism)
- DArT (or Diversity Arrays Technology)
- RAD markers (or Restriction site associated DNA markers)

There are 5 conditions that characterize a suitable molecular marker

1- Must be polymorphic

2- Co-dominant inheritance

3- Randomly and frequently distributed throughout the genome

4- Easy and cheap to detect

5- Reproducible

Molecular markers can be used for several different applications including

1- Germplasm characterization.

2- Genetic diagnostics.

3- Characterization of transformants.

4- Study of genome.

5- Organization and phylogenic analysis.

6- Paternity testing and the investigation of crimes.

7- Measure the genomic response to selection in livestock.

1- RFLP (Restriction fragment length polymorphism)

RFLPs: involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs.

A **RFLP:** occurs when the length of a detected fragment varies between individuals and can be used in genetic analysis.

Advantages:

1- Variant are co dominant

2- Measure variation at the level of DNA sequence, not protein sequence.

Disadvantage:

- Requires relatively large amount of DNA

2- AFLP (Amplified fragment length polymorphism)

In this analysis we can amplify restricted fragments and reduces the complexity of material to be analyzed (approx 1000 folds).it can be used for *comparison of closely related species only.*

Advantages:

1- Fast

2- Relatively inexpensive

3- Highly variable

Disadvantage:

1- Markers are dominant

2- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence.

3- RAPD (Random amplification of polymorphic DNA)

Random **A**mplification of **P**olymorphic **D**NA. It is a type of PCR reaction, but the segments of DNA that are amplified are random.

Advantages:

1- Fast

2- Relatively inexpensive

3- Highly variable

Disadvantage:

1- Markers are dominant

2- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence.

3- Data analysis more complicated

4- Micro satellite polymorphism, SSR or Simple sequence repeat

Microsatellites, Simple Sequence Repeats (SSRs), or Short Tandem Repeats (STRs), are repeating sequences of 1-6 base pairs of DNA.

Advantages:

1- Highly variable

2- Fast evolving

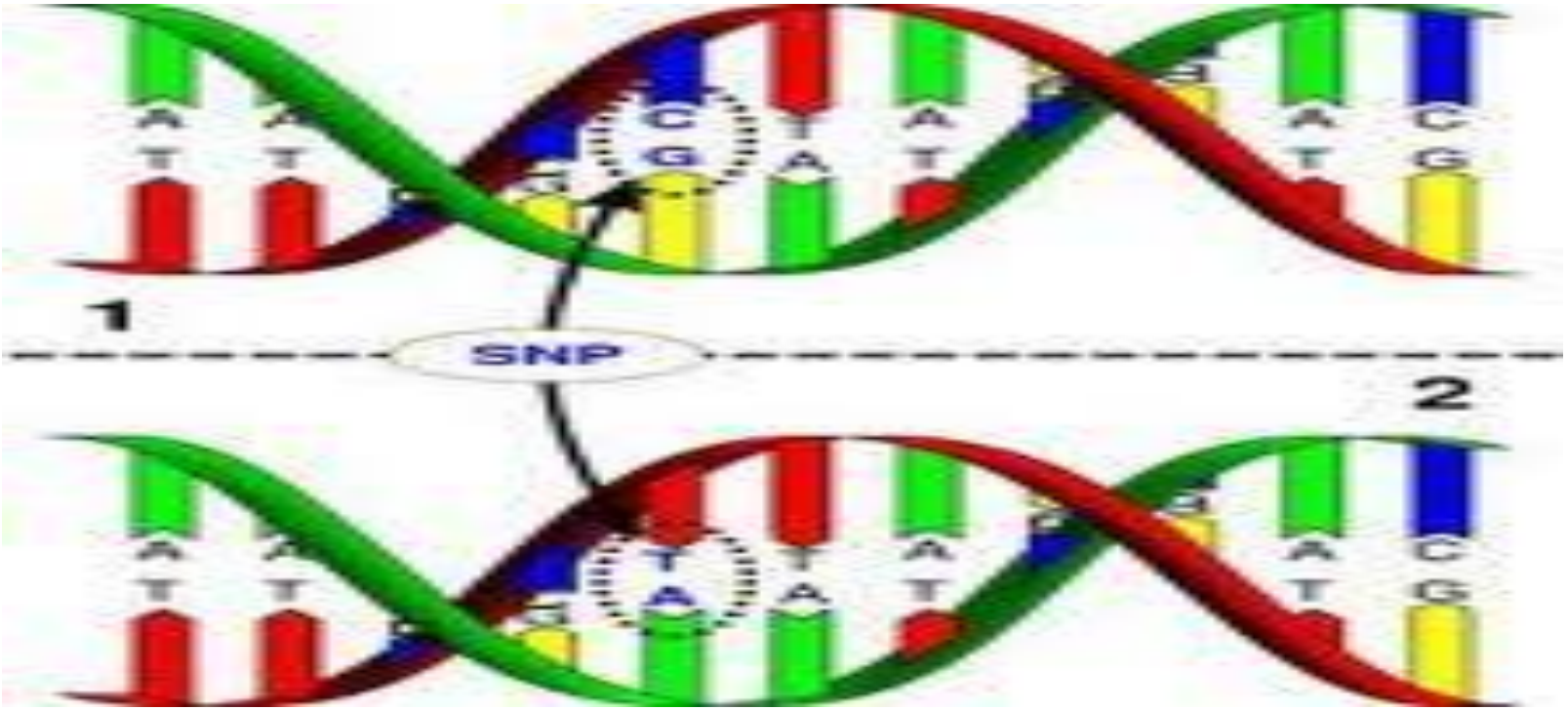
3- Co dominant

Disadvantage:

- Relatively expensive and time consuming to develop

5- SNP (or Single nucleotide polymorphism)

- A **single-nucleotide polymorphism** (**SNP**, pronounced *snip*) is a DNA sequence variation occurring when a single nucleotide — A, T,C, or G — in the genome (or other shared sequence) differs between members of a species or paired chromosomes in an individual.
- Used in biomedical research ,crop and livestock breeding programs.



6- STR (or Short tandem repeat)

- A **short tandem repeat** (STR) in DNA occurs when a pattern of two or more nucleotides are repeated and the repeated sequences are directly adjacent to each other.
- The pattern can range in length from 2 to 16 base pairs (bp) (for example $(CATG)_n$ in a genomic region) and is typically in the non-coding intron region
- Used in forensic cases.
- used for the genetic fingerprinting of individuals

Cancer markers

- The concept of tumour markers can mean anything that helps in the diagnosis of cancer
- In a more restricted sense it refers to the biochemical detection of cancer.

Detected by:

x-ray ; Ultra sound ; Computerised Tomography (CT) ; (Nuclear Medicine**)**

Magnetic resonance imaging (MRI) ; Gamma Camera and radioisotopes

Those detected on cancer tissue:

i) Biochemical methods : Oestrogen receptors in breast cancer

ii) Genetic methods : Mutated genes BRCA1 and BRCA2 in breast cancer

iii) Antibody methods : Carcinoembryonic antigen (CEA).

Detection by:

1- Biochemical techniques : steroids in adrenocortical cancer

2- Immunoassay : proteins, glycoproteins

Tumor marker conditions

1. A tumor marker should be present in or produced by tumor itself.

2. A tumor marker should not be present in healthy tissues.

3. Plasma level of a tumor marker should be at a minimum level in healthy subjects and in benign conditions.

4. A tumor marker should be specific for a tissue, it should have different immunological properties when it is synthesized in other tissues.

5. Plasma level of the tumor marker should be in proportion to the both size of tumor and activity of tumor.

6. Half life of a tumor should not be very long.

7. A tumor marker should be present in plasma at a detectable level, eventhough tumor size is very small

Potential uses of tumor markers

1- Screening in general population

2- Differential diagnosis of symptomatic patients

3- Clinical staging of cancer

4- Estimating tumor volume

5- As a prognostic indicator for disease progression

6- Evaluating the success of treatment.

7- Detecting the recurrence of cancer

8- Monitoring reponse to therapy

9- Radioimmunolocalization of tumor masses

10 In order to use a tumor marker for screening in the presence of cancer in asymptomatic individuals in general population, the marker should be produced by tumor cells and not be present in healthy people.

11- However, **most tumor markers are present in normal, benign and cancer tissues and are not spesific enough to be used for screening cancer**.

12- Few markers are specific for a single individual tumor, most are found with different tumors of the same tissue type.

13- They are present in higher quantities in blood from cancer patients than in blood from both healthy subjects and patients with benign diseases.

14- Some tumor markers have a plasma level in proportion to the size of tumor while some tumor markers have a plasma level in proportion to the activity of tumor.

15- The clinical staging of cancer is aidded by quantitiation of the marker.

16- Serum level of marker reflects tumor burden.

17- The level of the marker at the time of diagnosis may be used as a prognostic indicator for disease progression and patient survival. After successful initial treatment, such as surgery, the marker value should decrease. The rate of the decrease can be predicted by using the half life of the marker.

18- The magnitude of marker reduction may reflect the degree of success of the treatment.

19- In the case of recurrence of cancer, marker increases again.

20- Most tumor marker values correlate with the effectiveness of treatment

Tumor markers can be classified by type of molecule

1- ENZYMES

A- Alkaline Phosphatase (ALP)

Increased alkaline phosphatase activities are seen in primary or secondary liver cancer. Its level may be helpful in evaluating metastatic cancer with bone or liver involvement. Placental ALP, regan isoenzyme, elevates in a variety of malignancies, including ovarian, lung, gastrointestinal cancers and Hodgkin's disease.

B- Prostatic acid phosphatase (PAP)

It is used for staging prostate cancer and for monitoring therapy. Increased PAP activity may be seen in osteogenic sarcoma, multiple myeloma and bone metastasis of other cancers and in some benign conditions such as osteoporosis and hyperparathyroidism.

C- Prostate Specific Antigen (PSA)

The clinical use of PAP has been replaced by PSA. PSA is much more specific for screening or for detection early cancer. It is found in mainly prostatic tissue.

PSA exists in two major forms in blood circulation. The majority of PSA is complexed with some proteins. A minor component of PSA is free.

PSA testing itself is not effective in detecting early prostate cancer. Other prostatic diseases, urinary bladder cateterization and digital rectal examination may lead an increased PSA level in serum.

The ratio between free and total PSA is an reliable marker for differentiation of prostatic cancer from benign prostatic hyperplasia.

The use of PSA should be together with digital rectal examination and followed by transrectal ultrasonography for an accurate diagnosis of cancer.

Serum level of PSA was found to be correlated with clinical stage, grade and metastasis

- The greatest clinical use of PSA is in the monitoring of treatment.
- This treatment includes radical prostatectomy, radiation therapy and antiandrogen therapy.
- The PSA level should fall below the detection limit.
- This may require 2-3 weeks. If it is still at a high level after 2-3 weeks, it must me assumed that residual tumor is present.
- Androgen deprivation therapy may have direct effect on the PSA level that is independent of the antitumor effect. **This subject must be considered always.**

2- HORMONES

A- Calcitonin

- Calcitonin is a hormone which decreases blood calcium concentration.
- Its elevated level is usually associated with medullary thyroid cancer.
- Calcitonin levels appear to correlate with tumor volume and metastasis.
- Calsitonin is also useful for monitoring treatment and detecting the recurrence of cancer.
- However calcitonin levels are also at a high levels in some patients with cancer of lung, breast, kidney, liver and in nonmalignant conditions such as pulmonary diseases, pancreatitis, Paget's disease, hyperparathyroidism, myeloproliferative disordes and pregnancy.

B- Human Chorionic Gonadotropin (hCG)

It is a glikoprotein appears in pregnancy. Its high levels is a useful marker for tumors of placenta and some tumors of testes.

hCG is also at a high level in patients with primary testes insufficiency.

hCG does not cross the blood-brain barier. Higher levels in BOS may indicate metastase to brain.

3- ONCOFETAL ANTIGENS

A- Carcinoembryonic antigen (CEA):

It is a cell-surface protein and a well defined tumor marker.

CEA is a marker for colorectal, gastrointestinal, lung and breast carcinoma.

CEA levels are also elevated in smokers and some patients having benign conditions such as cirrhosis, rectal polips, ulcerative colitis and benign breast disease.

CEA testing should not be used for screening. Some tumors don't produce CEA. **It is useful for staging and monitoring therapy.**

B- α-Fetoprotein (AFP):

α-fetoprotein is a marker for hepatocellular and germ cell carcinoma.

It is also increased in pregnancy and chronic liver diseases.

AFP is useful for screening (AFP levels greater than 1000 µg/L are indicative for cancer except pregnancy), determining prognosis and monitoring therapy of liver cancers.

AFP is also a prognostic indicator of survival.

Serum AFP levels is less than 10 µg/L in healthy adults. Elevated AFP levels are associated with shorter survival time.

AFP and hCG combined are useful in classifying and staging germ cell tumors.One or both markers are increased in those tumors.

3- CARBONHYDRATE MARKERS

- These markers either are antigens on the tumor cell surface or are secreted by tumor cells.
- They are high-molecular weight mucins or blood group antigens. Monoclonal antibodies have been developed against these antigens.
- Most reliable markers in this group are CA 15-3, CA 125 and CA19-9.

A- CA 15-3

- CA 15-3 is a marker for breast carcinoma. Elevated CA 15-3 levels are also found in patients with pancreatic, lung, ovarian, colorectal and liver cancer and in some benign breast and liver diseases.
- **It is not useful for diagnosis.** It is most useful for monitoring therapy.

B- CA 125:

Although CA 125 is a marker for ovarian and endometrial carcinomas, it is not specific. CA 125 elevates in pancreatic, lung, breast, colorectal and gastrointestinal cancer, and in benign conditions such as cirrhosis, hepatitis, endometriosis, pericarditis and early pregnancy.

It is useful in detecting residual disease in cancer patients following initial therapy.

A preoperative CA 125 level of less than 65 kU/L is associated with a greater 5 y survival rate than is a level greater 65 kU/L.

It is also useful in differentiating benign from malignant disease in patients with ovarian masses.

In the detection of recurrence, use of CA 125 level as an indicator is about 75 % accurate.

C- CA 19-9

CA 19-9 is a marker for both colorectal and pancreatic carcinoma.However elevated levels were seen in patients with hepatobiliary, gastric, hepatocellular and breast cancer and in benign conditions such as pancreatitis and benign gastrointestinal diseases.

CA 19-9 levels correlate with pancreatic cancer staging.

It is useful in monitoring pancreatic and colorectal cancer.

Elevated levels of CA 19-9 can indicate recurrence before detected by radiography or clinical findings in pancreatic and colorectal cancer.

4- PROTEIN MARKERS

A- β_2-microglobulin:

β_2-microglobulin is a marker for multiple myeloma, Hodgkin lymphoma. It also increases in chronic inflammation and viral hepatitis.

B- Ferritin:

Ferritin is a marker for Hodgkin lymphoma, leukemia, liver, lung and breast cancer.

C- Thyroglobulin:

It is a useful marker for detection of differentiated thyroid cancer.

D- Immunoglobulin:

Monoclonal immunoglobulin has been used as marker for multiple myeloma for more than 100 years.

Monoclonal paraproteins appear as sharp bands in the globulin area of the serum protein electrophoresis.

Bence-Jones protein is a free monoclonal immunoglobulin light chain in the urine and it is a reliable marker for multiple myeloma.

5- RECEPTOR MARKERS

- Estrogen and progesterone receptors are used in breast cancer as indicators for hormonal therapy.
- Patients with positive estrogen and progesterone receptors tend to respond to hormonal treatment.
- Those with negative receptors will be treated by other therapies.
- Hormone receptors also serve as a prognostic factors in breast cancer. Patients with positive receptor levels tend to survive longer.
- Cytoplasmic estrogen receptors are now routinely measured in samples of breast tissue after surgial removal of a tumor. Of patients with breast cancer, 60 % have tumors with estrogen receptor.
- Approximately two thirds of patients with estrogen receptor (+) tumors respond to the hormonal therapy. 5% of patients with estrogen receptor (-) tumors respond to the hormonal therapy.

- Progesterone receptor testing is a useful adjunt to the estrogen receptor testing. Because progesterone receptor synthesis appears to be dependent on estrogen action.
- Measurement of progesterone receptors provides a confirmation that all the steps of estrogen action are intact. Indeed breast cancer patients with both progesterone and estrogen receptor (+) tumors have a higher response rate to hormonal therapy.
- **C-erbB2 (HER-2 Neu):**
- It is receptor for epidermal growth factor (EGF) but it doesn't contain EGF binding domain. It serves as a co-receptor in EGF action
- In the case of increased expression of C-erbB2 leads the oto-activation and increased signal transduction.
- Increased expression of C-erbB2 was determined in some cancers. It was suggested as an important factor for carcinogenesis and metastasis
- Routine measurement of C-erbB2 was started in our hospital

6- GENETIC CHANGES

Four classes of genes are implicated in development of cancer:

1) **Protooncogenes** which are responsible for normal cell growth and differentiation

2) **Tumor suppressor genes** which are involved in recognition and repair of damaged DNA.

3) **Apoptosis-related genes** are responsible for regulation of apoptosis

4) **DNA repair genes**

- Alterations on these genes may lead tumor development.

Susceptible protooncogenes:

K-ras, N-ras mutations are found to be correlated acute myeloid leukemia, neuroblastoma

Susceptible DNA repair genes:

BRCA1 and BRCA2 are specific genes in inherited predisposition for developing breast and over cancer, and mutations on these genes are newly measured in some laboratories.

- Mismatch-repair genes are mutated in some colon cancers.

Susceptible tumor suppressor genes:

- Retinoblastoma gene
- P53 gene
- P21 gene
- Those genetic markers are very new and not routinely measured in laboratories.

Chromosomal translocation:

- c-myc gene has been found to be translocated from 8.chromosom to 14. chromosom and than become activated in Burkitt's lymphoma.
- myc gene encodes a DNA-binding protein which stimulates cell dividing.

What is Bioinformatics?

Is the development and use of computer applications for the Analysis, Interpretation, Simulation and Prediction of biological Systems and corresponding experimental methods in nature sciences.

Offers an ever more essential input to:

1- Molecular Biology

2- Pharmacology (drug design)

3- Agriculture

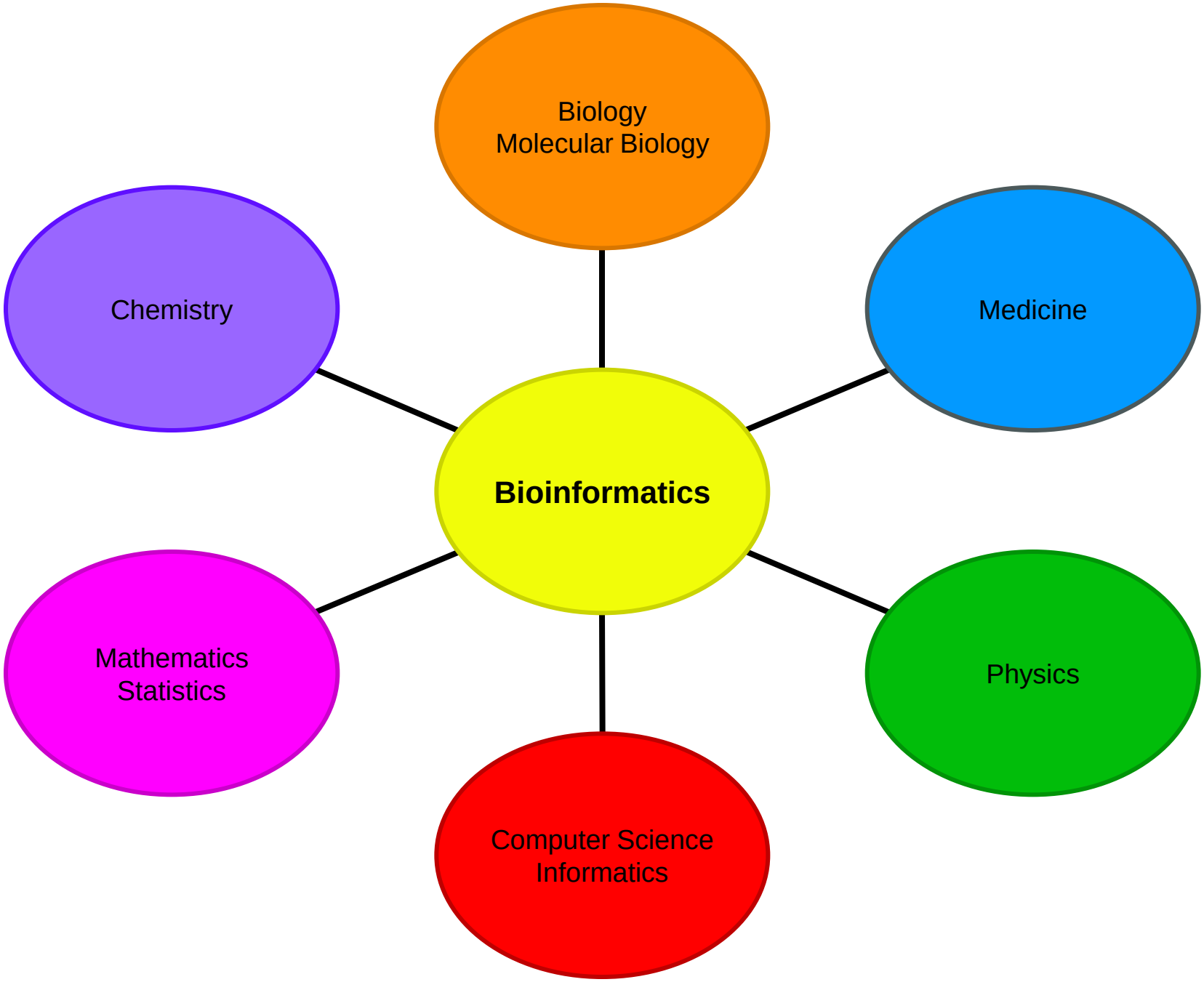
4- Biotechnology

5- Clinical medicine

6- Anthropology

7- Forensic science

8- Chemical industries (detergent industries, etc.)


Biology
Molecular Biology
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Medicine
Bioinformatics
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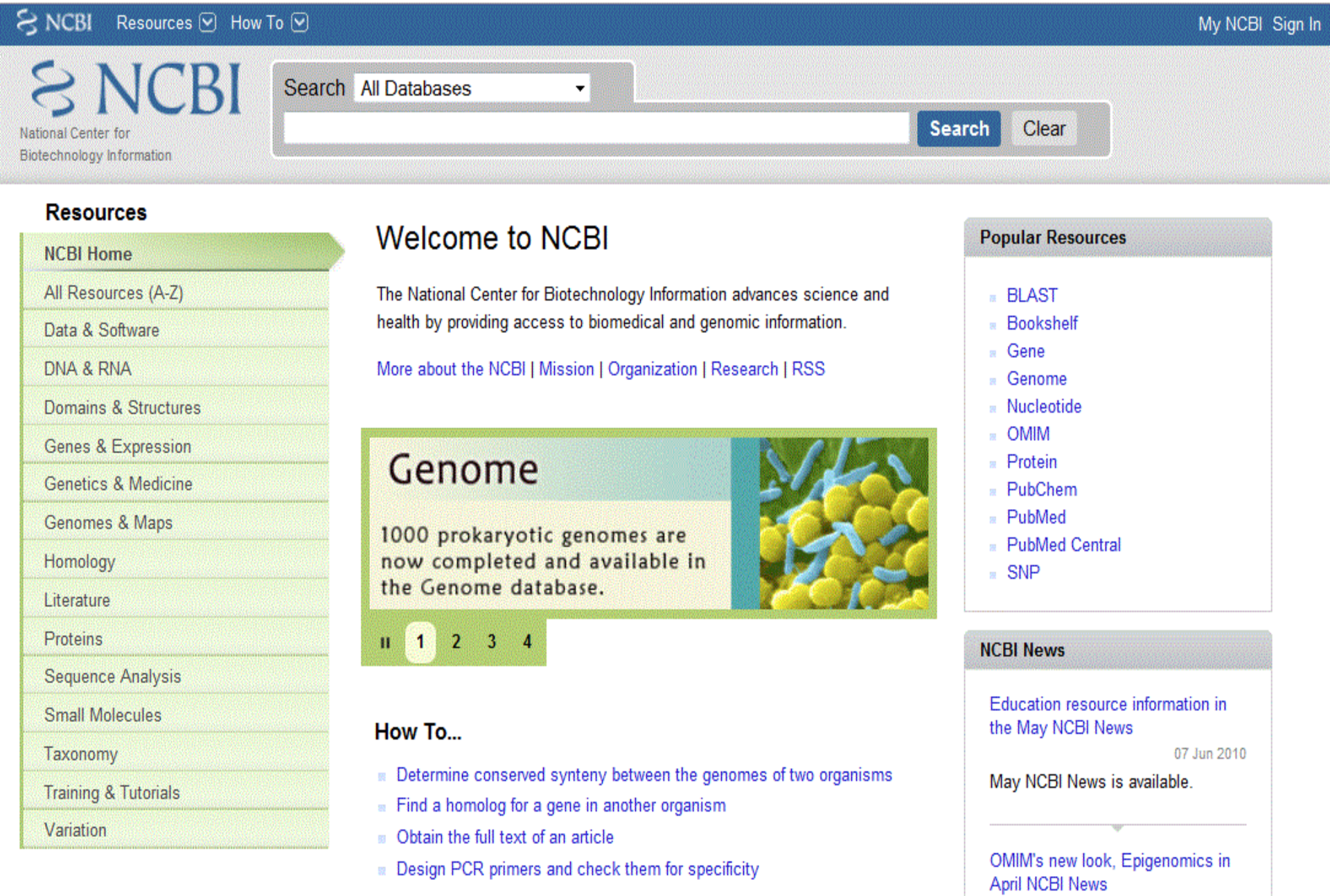
Example for Bioinformatics is National Center for Biotechnology Information (NCBI)



NCBI Resources How To

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Search

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GSS
EST
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Gene

Resources

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- Data & Software
- DNA & RNA
- Domains & Structures
- Genes & Expression
- Genetics & Medicine

BI

chnology Information advances science and biomedical and genomic information.

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ADVANTAGES OF BIOINFORMATICS:

1- More relevant *inter-related* information in one place.

2- Makes it easier to find additional relevant information related to your initial query.

3- Potentially find information *indirectly* linked, but *relevant* to your subject of interest.

4- Easier to build a ‘story’ based on *multiple* pieces of biological evidence.