Genetics of breast cancer

Introduction

- **Breast cancer is the second cause of cancer-related death in women**
- **More than 200,000 breast cancer women is diagnosed each year**
- **Out of these 200,000 women, 40,000 died of this disease**

Main risk factors:

Age	increases risk after age 30
Family history	1st degree relative with: premenopausal + bilateral breast cancer
Nulliparous	A state of never having delivered a child
Menstrual history	• early age at menarche (less than 12 years) • OR late age at menopause (more than 55 years)
Benign breast diseases	such as proliferative disease with atypical hyperplasia

Other risk factors (Check the pathology lecture for more):

Exogenous estrogen - Oral contraceptives – Obesity - Alcohol consumption - Cigarette smoking - High fat diet

Genetics and family history of breast cancer

- About 5-10 % of breast cancers are related to specific inherited mutations

When do we suspect that this breast cancer is genetics?

1. Developing breast cancer before menopause
2. Having bilateral cancer
3. Having other associated cancers (e.g.: Ovarian cancer)
4. Having 1st degree family relatives with breast cancer in premenopausal age

Gene mutations (BRCA1 and BRCA2):

- both of these two genes are thought to function in DNA repair
- They act as Tumor Suppressor genes

Gene mutation	Location	Popularity of women with hereditary BC*	Other cancer risks
BRCA1	On chromosome 17q21.3	Half	May lead to ovarian cancer
BRCA2	On chromosome 13q12-13	One-third	May lead to ovarian cancer (more than BRCA1)

*Breast cancer

- **Gene mutations (BRCA1 and BRCA2) "continued":**

- most carriers of these mutations will develop breast cancer by the age of 70 years
- as compared with only 7% of women who do not carry a mutation will develop a breast cancer
- Less common genetic diseases associated with breast cancer:
  - Li-Fraumeni syndrome (germ-line mutations in p53
  - Cowden disease (germ-line mutations in PTEN)
  - Carriers of the ataxia-telangiectasia gene

Assessment of breast cancer

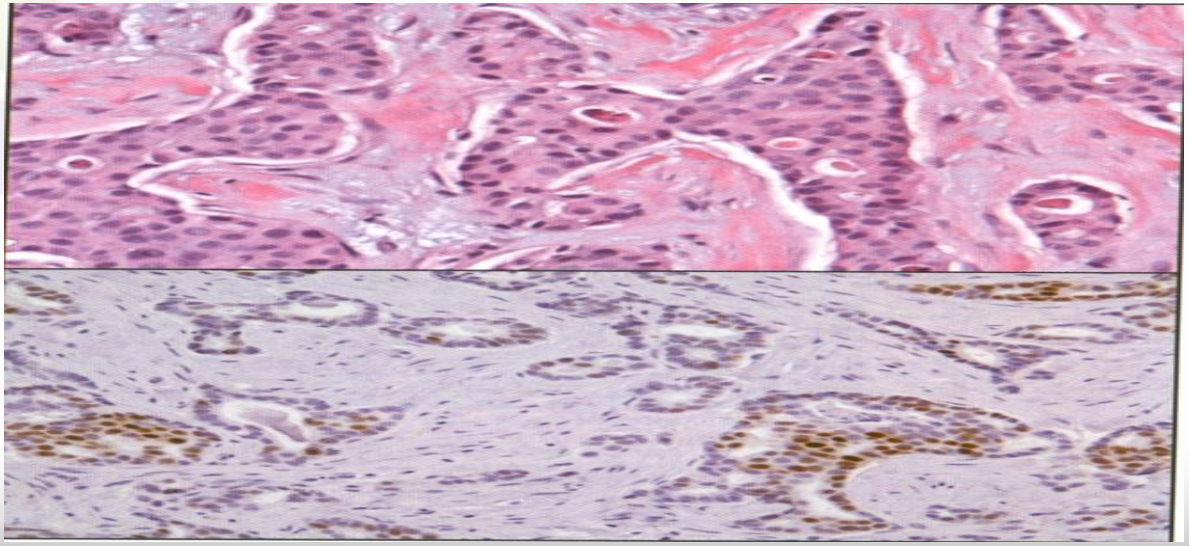
- **Prognostic factors:** (when a physician is facing a breast cancer, he has to check the followings to determine the prognosis)

✓ Tumor size
✓ Histologic type
✓ Tumor grade
✓ Lymph node staging
✓ Evidence of vascular or lymphatic invasion

- **Predictive factors:** (to be able to choose the optimal therapy for the patient)

1. Hormone receptors:
   - ✓ Around 60-70% of breast carcinomas express Estrogen receptors (ERs) and progesterone receptors (PRs)
   - ✓ Demonstrated by Immunohistochemistry
   - ✓ Furthermore, tumors that express these receptors depend on estrogen, progesterone or both for growth
   - ✓ So, It's easy to treat this breast cancer type by antagonists these receptors, such as:
     1. Tamoxifen
     2. Aromatase inhibitors

Normal



Immunohistochemistry of ERs (brown staining)

Molecular Prognostic and Predictive Factors

- **Human Epidermal Growth Factor Receptor 2 (HER2) Tyrosine Kinase:**
  - In normal cells, there is one copy of the HER2 gene on each chromosome 17.
  - It transmits signals regulating cell growth and survival.
  - In approximately %15 to %25 of breast cancer, HER2 gene is found to be amplified
    (from 2-fold to more than 20-fild in each tumor nucleus relative to CHR17)
  - HER2-Positive breast cancer is correlated with unfavorable pathologic tumor characteristics, such as:
    1. Larger tumor size
    2. Positive Axillary nodes
    3. Higher nuclear grade
    4. Higher proliferative index

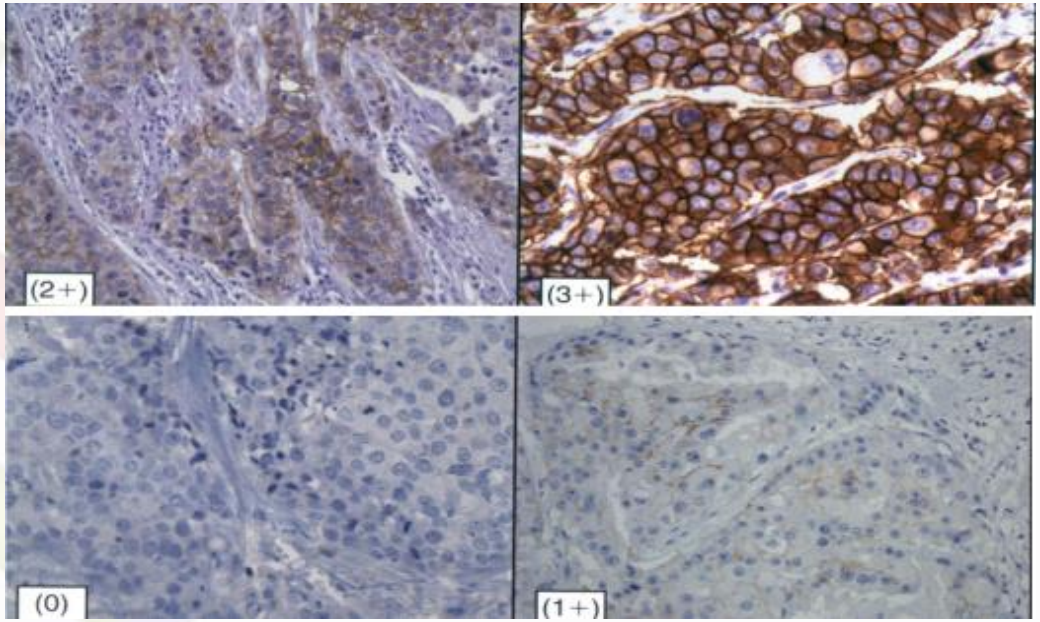
- **The Herceptin Molecule (Trastuzumab):**
  - Herceptin is the drug used against HER2-positive tumor cells.
  - High specificity and affinity for the HER2 protein.
  - It's a molecular genetic targeted therapy, so we don't have to give cytotoxic drugs.

- **Demonstration of HER2 neu receptors can be done by using the following techniques:**
  1. **Immunohistochemistry:**
     Antibodies against HER2 receptors are applied to the tissue and if the antigen (HER2) is present a reaction is visualized by means of a dye or a color-producing-enzyme which is used to label the antibody.

  2. **Fluorescent or Silver in situ hybridization (FISH or SISH)**

Both will be explained in the next few slides

1. Immunohistochemistry:



Patient 0:

Negative HER2/neu, She will no benefit from Herceptin.

Patient +1:

Negative HER2/neu, very weak stain (less than 10%, scattered), a (+1) is not important, it is regarded as "negative" (at least 10% of the cells should be stained in order to use herceptin).

Patient +2:

About 15%-20% of cells are stained. She could be Positive or Negative HER2/neu, we need to do FISH stain to confirm.

Patient +3:

Positive, treat with Herceptin.

2. Fluorescent or Silver in situ hybridization (FISH or SISH):

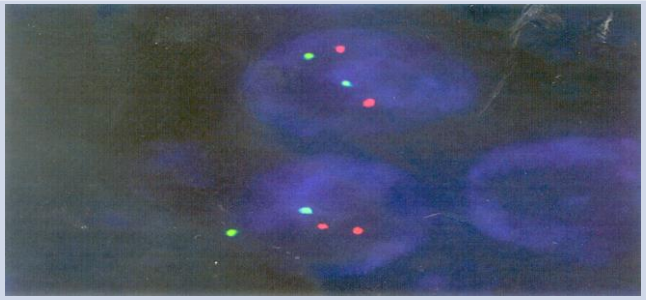
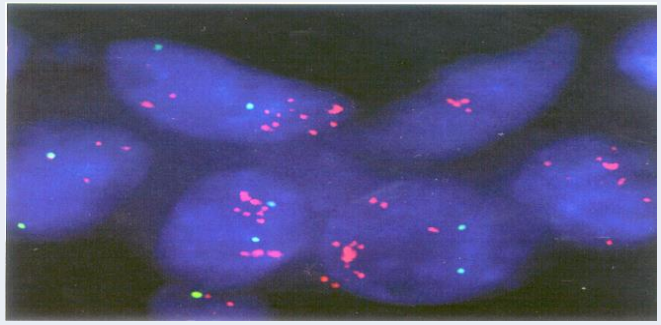
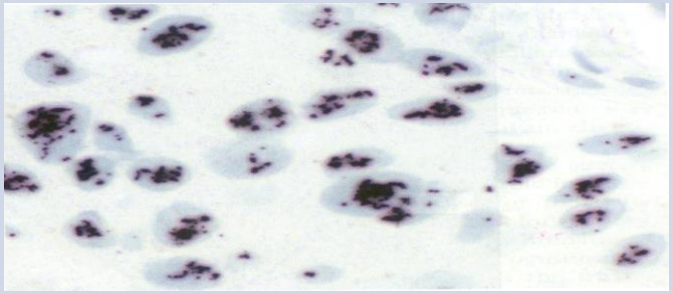
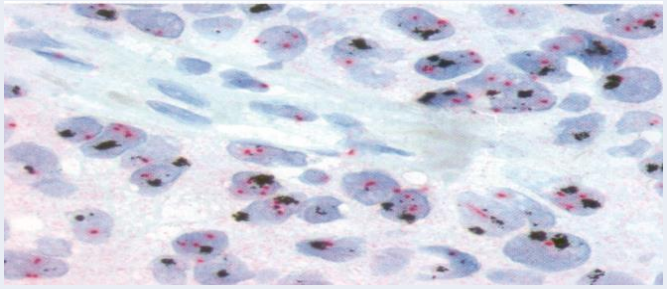
- In FISH, fluorescently tagged DNA or RNA probes are used to identify genomic sequences of interest.
- FISH provides high resolution for identification of specific abnormalities, e.g., gene amplification, deletions, and translocations.

- Principles of hybridization

✓ DNA is double stranded.
✓ Bonds between complementary bases hold strands together (Cytosine & Guanine; Adenine & Thymine).
✓ Heat/alkalinise DNA – separation of strands ('denaturation') occurs.
✓ Cool separated strands – complementary double strands re-form.
✓ Labelled complementary single-strand DNA can identify a DNA sequence (e.g. a gene) in intact cells or disrupted cell preparations.

FISH requires denaturation, hybridization with a probe, and washing.
First, a probe specific for the target of interest is applied to the slide, along with a nuclear counterstain and reagents or heat that enhance denaturation of target DNA and reduce background.
The slides are sealed and incubated in a humid environment under conditions that denature the DNA, allowing hybridization to occur between the probe and its cDNA sequence. The unbound probe is then removed by washing, and patterns of fluorescence are interpreted by fluorescence microscopy

This video here is less than 2 minutes long but it'll help you understand how FISH works, so if you find this slide a bit confusing, we HIGHLY RECCOMENED watching it:
https://www.youtube.com/watch?v=nm8Ai1CI9Is
(REMEMBER!! IT'S LESS THAN 2 MINUTES LONG!! WATCH IT!!)

Image	Comment
	Green = Chromosome 17 Red = HER2/NEU This image shows no HER/NEU gene amplification
	This image shows HER2/NEU gene mutation and amplification. (More than 2 Copies of HER2/NEU) Herceptin (Transtuzumab) is used for treatment
	Silver in situ hybridization image.
	Dual-color silver in situ hybridization (SISH) image.

Molecular subtypes of Breast Cancer

(Not found in the handouts/slides, but Dr. Ammar mentioned them)
Source: Robbins Basic Pathology, page: 709

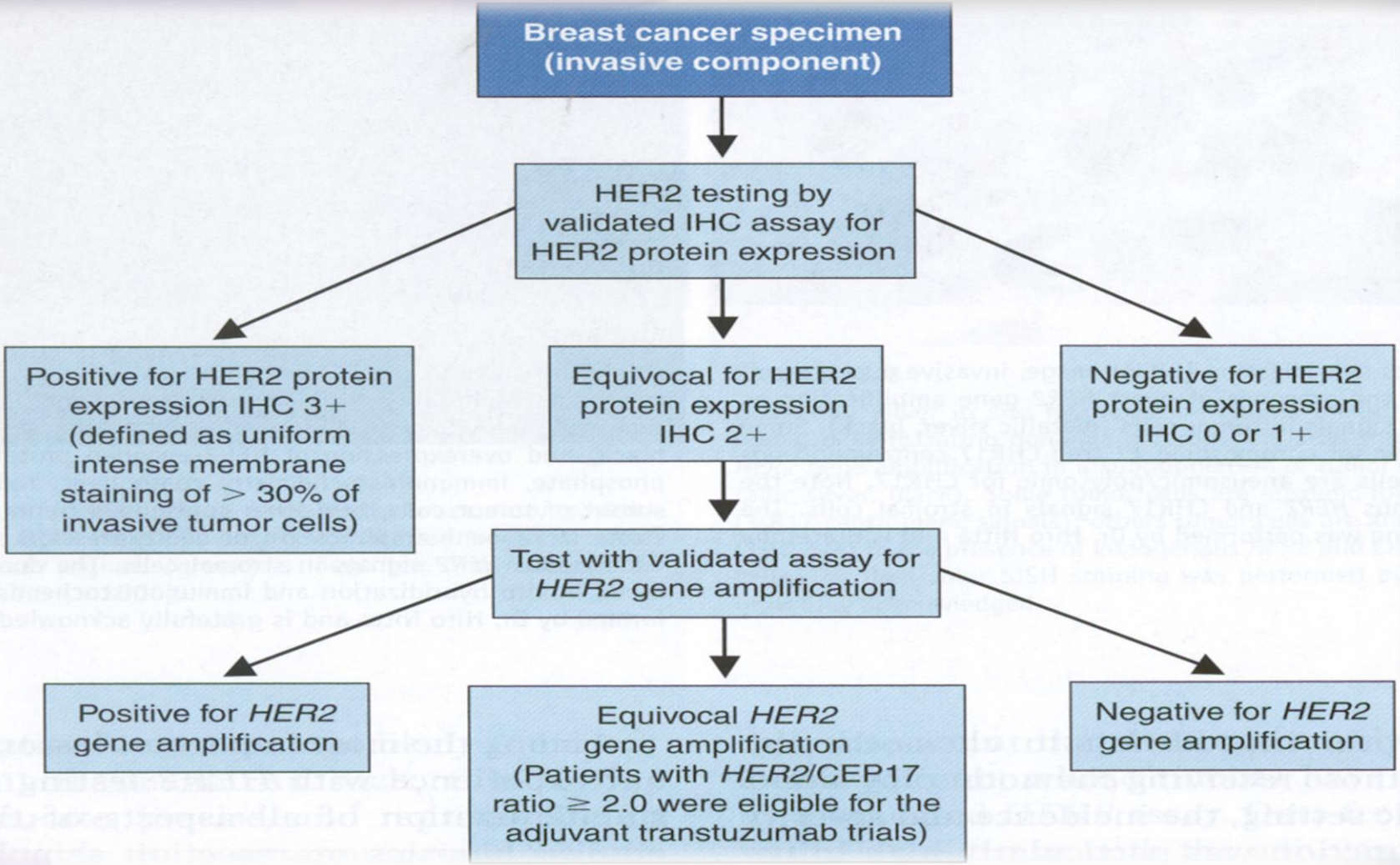
- **Gene expressing profiling can separate breast cancer into four molecular subtypes:**

1. **Luminal A: estrogen receptor-positive, HER2/NEU-negative**
   (Tamoxifen will be effective)

2. **Luminal B: estrogen receptor-positive, HER2/NEU- overexpressing(=positive)**
   (Tamoxifen and Herceptin both will be effective)

3. **Luminal C: estrogen receptor-negative, HER2/NEU-overexpressing(=positive)**
   (Herceptin will be effective)

4. **Basal-Like or "Triple negative": estrogen receptor-negative, HER2/NEU-negative**
   It's called "Tripe Negative" because it's negative for Estrogen receptors, Progesterone receptors, and HER2/NEU.
   This tumor is very bad and aggressive.



ASCO/CAP guideline recommendations for the optimal algorithm for HER2 testing by IHC

MCQs

1. Which of the following criteria is associated with GENETIC breast cancer?
   a. Happens after menopause
   b. Usually unilateral
   c. Associated with ovarian cancer
   d. Both a & c
2. BRCA2 gene is a DNA repairer gene. In which chromosome is it located?
   a. 17q21.3
   b. 17q12-13
   c. 13q21.3
   d. 13q12-13
3. HER2 gene is located at?
   a. Chromosome 13
   b. Chromosome 15
   c. Chromosome 17
   d. Chromosome 19
4. A 33-year-old female present to the obstetrician/gynecologist with a pabable mass in her left breast. From her history her mother died from breast cancer at the age of 38. The doctor arranged some investigations and found postivie-estrogen-receptor in the carcinogenous cells. What is the most approprite drug used in this case?
   a. Herceptin
   b. FISH or SISH
   c. Aromatase inducers
   d. Tamoxifen

ANS: 1.c 2.d 3.c 4.d

MCQs

5. A young-female patient came to the clinic after she felt some palpable masses in the axillary area. Clinical examinations showed: large tumor size in both of her breasts & positive axillary lymph nodes. From histopathology report: high nuclear grade & high proliferative index. The doctor administered some oral drugs and chemotherapy and endocrine therapy. She responded well. What is the most likely diagnosis?

a. Breast cancer with over-expression of HER2 gene
b. Breast cancer caused by estrogen-receptor gene.
c. Luminal a breast cancer.
d. Triple negative

6. A 33-year-old female present to the obstetrician/gynecologist with pabable mass in her left breast. From her history her mother died from breast cancer at age of 38. The doctor arranged some investigations and she found postive HER2 gene amplification in the carcinogenous cells.
What is the most approprite drug used in this case?

a. Herceptin
b. FISH or SISH
c. Aromatase inhibitors
d. Tamoxifen

ANS: 5.a 6.a


BEST WISHES!!!