MEDICINE
KING SAUD UNIVERSITY

تمت المراجعة
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HUMAN GENETICS

Genetics of Breast Cancer

- ✓ Notes
- ✓ Important
- ✓ Extra

Objectives

I. Recognize carcinoma of the female Breast as the leading cause of cancer morbidity and mortality among women.

II. Know the risk factors of breast cancer with special emphasis on the genetics and importance of family history.

III. Know the role of molecular prognostic and predictive factors in breast cancer with special emphasis on hormonal receptors and Her2-neu status.

What is a Gene?

A gene is the basic physical and functional unit of heredity. Genes, which are made up of DNA, act as instructions to make molecules called proteins.

Every person has two copies of each gene, one inherited from each parent.

Regions of the Gene:

Genes have three regions, the promoter, coding region, and termination sequence. The promoter turns the gene on. The coding region has the protein building information, and the termination sequence indicates the end of a gene.

Mutations:

DNA replication is to an extent extremely accurate, but errors can occur during this process and those errors are called *"Mutations".* Once those mutations happen, many cellular functions will be affected and in turn, many functions in our body will be affected too; *cancer can be due to genetic mutations.*

Cancer and Genetics (EXTRA)

Cancer is a genetic disease, cancer is caused by certain changes to genes that control the way our cells function, especially how they grow and divide.

Patterns of genetic changes causing cancer:

Inherited changes

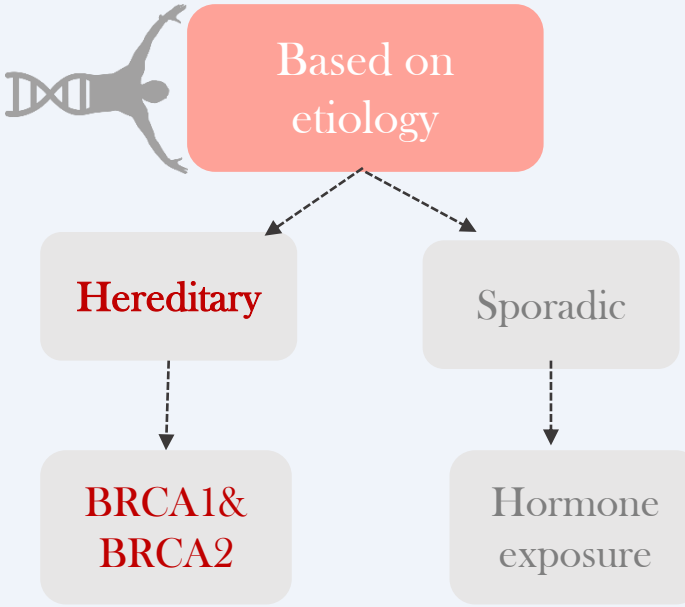
Acquired changes

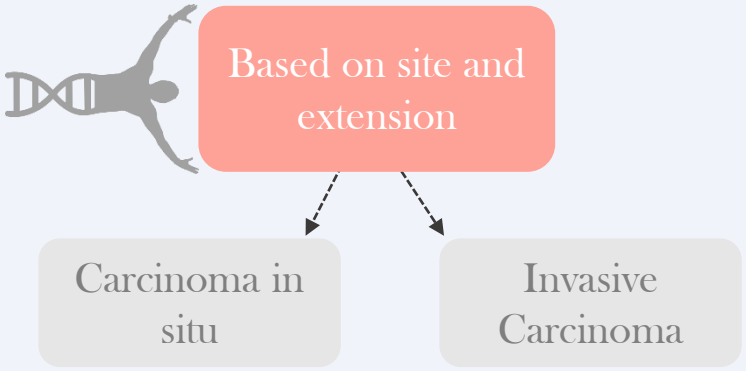
In general, cancer cells have more genetic changes than normal cells. But each person's cancer has a unique combination of genetic alterations. Some of these changes may be the result of cancer, rather than the cause. As the cancer continues to grow, additional changes will occur. Even within the same tumor, cancer cells may have different genetic changes.

Key points about breast cancer: (more details in Pathology lecture; check our team)

- Breast cancer is the most common cancer among women.
- Symptoms include a lump or thickening of the breast, and changes to the skin or the nipple.
- Risk factors can be genetic, but some lifestyle factors, such as alcohol intake, make it more likely to happen.
- A range of treatments is available, including surgery, radiation therapy, and chemotherapy.
- Many breast lumps are not cancerous, but any woman who is concerned about a lump or change should see a doctor.

Classification of Breast cancer:





Genes involved in Breast Cancer

- About 5% to 10% of breast cancers are related to specific inherited mutations. (Read it only)

Those mutations happen in those genes:

BRCA1& BRCA2 — ERs & PRs — HER 2

- Before genetic revolution, all types of breast cancer were treated by the same method; chemotherapy.
- Familial cases of breast cancer –BRCA mutations- are treated by breast removal "mastectomy".
- BRCA1 and BRCA2 are strong indicators of ovarian cancer as well.
- ERs and PRs positive patients are treated by hormonal therapy.
- HER2 is a growth factor receptor; treated with monoclonal antibodies that block those mutated receptors.
- It is VERY IMPORTANT to know the genetic profile of breast cancer patients to know how to treat them since different genes are treated differently.

BRCA's gene:

The name "BRCA" is an abbreviation for "BReast CAncer gene."

BRCA1 and BRCA2 are human genes that produce **tumor suppressor proteins**. These proteins help **repair damaged DNA** and, therefore, play a role in ensuring the stability of each cell's genetic material. When either of these genes is mutated, or altered, such that its protein product is not made or does not function correctly, DNA damage may not be repaired properly. As a result, cells are more likely to develop additional genetic alterations that can lead to cancer.

- BRCA1 and BRCA2 which are mutated in familial breast cancers are involved in DNA repair.
- BRCA1 is more aggressive than BRCA2.

Location of these genes:

- BRCAI is located on chromosome 17q 21.3
- BRCA2 is located on chromosome 13q 12-13.

You only have to know that BRCA1 is located on chromosome 17 and BRCA2 on chromosome 13.

Epidemiology of this familial mutation: (Read it only)

Most carriers of those mutant genes will develop breast cancer by the age of 70 years, as compared with only 7% of women who do not carry a mutation.

- Being positive for those two genes put you at really high risk ,so we have to do both hysterectomy and mastectomy but if you want to delay it for any purpose you must be followed up regularly.

Estrogen (ERs) and Progesterone (PRs) receptors

- 60% to 70% of breast carcinomas express estrogen receptors (ERs) and progesterone receptors (PRS).
- The presence of ERS in breast cancer is a weak prognostic factor, however, it is optimally useful as a predictive factor for the benefit of adjuvant tamoxifen or aromatase inhibitor therapy.

- Most of the types of breast cancers are hormonal dependent, they depend on their growth on estrogen and progesterone, why?

Because the cancer cells have receptors on their nuclei and their cytoplasmic membrane which are under the influence of estrogen.
So if you cut the source of estrogen from them the tumor will shrink.

- Normal breast have estrogen receptors but in case of a cancer it would be over expressed.
- It is also common in obese women. Why? Because fat cells produce estrogen in small amounts.
- Patients with HER 2 mutations used to be on Tamoxifen for 5 years but now it is exceeded to 10 years!

Case Scenario (Important)

Case Scenario

Lets now take a scenario about a woman called Badriah:

بدرية هذي راحت اشترت مجلة سيدتي ولقت على الغلاف عنوان خوفها (سرطان الثدي يفتك بالنساء) , فقررت تروح تفحص وجت لباص فحص سرطان الثدي اللي عندنا برى المستشفى , ممتاز؟ , سوولها Screening **وهي الخطوة الاولى.**

لقواعندها Irregular area with calcification in the mammogram .

عادة يبدأ الموضوع كذا , بعد كذا اخذوا منها Biopsy وطلع عندها Ductal carcinoma of the Breast .

- It is important to see calcification in the mammogram, in a benign breast we can see calcification, but the calcification in the breast cancer it is usually with a unique shape.
- Most of the breast cancers arise from the duct of the breast, and some from the lobules or the tubules, the one arising from the ducts is called ductal carcinoma of the breast.

The case continues...

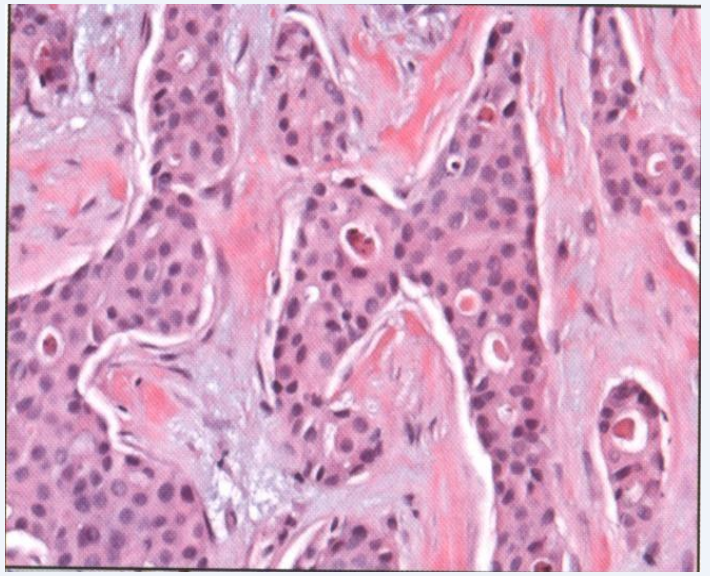
Case Scenario

2nd step in this case :

The biopsy specimen is taken to the lab.

- In the breast cancer grading (degree of differentiation) we look for three criteria:

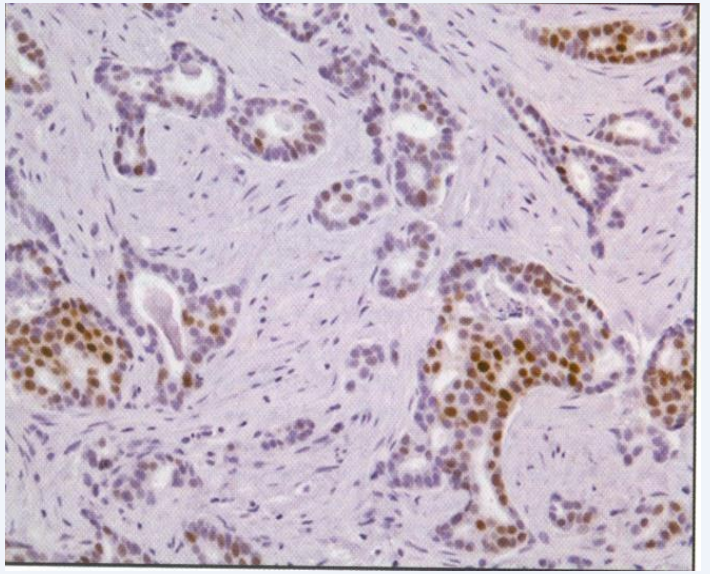
1. Is the tumor producing **tubules**? The more the tubules the better the degree of differentiation.
2. The degree of **pleomorphism** (variation in the size and the shape of the cells).
3. The degree of **mitosis**.



3rd Step in this case :

After that we look for **estrogen receptors** in the tumor cells , we can look for it by immunohistochemistry with a stain , if she has positive receptors it should look brown (as in this picture) , so we can give her **Tamoxifen** for the rest of her life.

- If the patient has ER, PR and HER2 positive then it means Luminal B. (Exam will be case scenario)



HER2 Gene

HER2 Gene (Also called HER2-neu)

Normal cells have one copy of the HER 2 gene on each chromosome 17 (CHR17) and when this gene is expressed in normal epithelial cells, **it is transmits signals regulating cell growth and survival.**

In approximately 15% to 25% of breast cancer, the HER2 gene is found to be amplified 2 folds to greater than 20 folds in each tumor cell nucleus.

As a result, HER2 positive breast cancers tend to be aggressive. (The most aggressive breast cancer). Also, triple negative** has a really bad prognosis.

HER2-neu Gene:

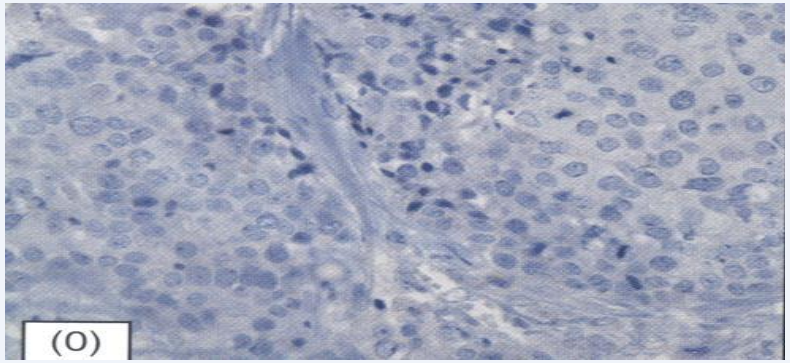
- This gene is present on the cytoplasmic membrane of the cell, and it produce some proteins that enhance the proliferation of the cell, sometimes in the cancer cells, this gene is over expressed.
- **A patient with a mutation in this gene would present later with a possible lymph node metastasis.**
- **Triple negative is basically when ER, PR and HER2 are all negative. The only option of treatment is chemotherapy and it is not really effective ☹

Treatment for Her2 Gene Mutation :

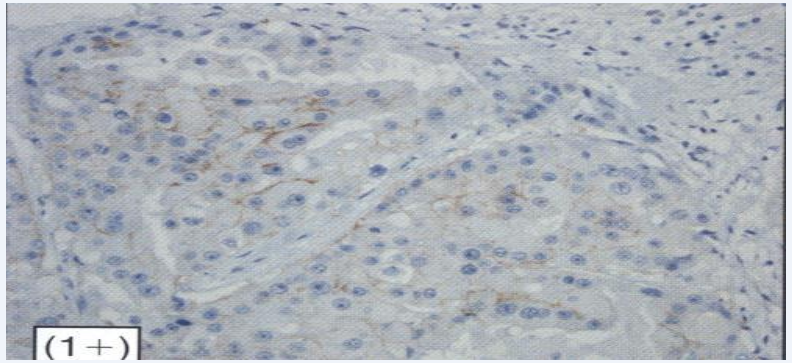
The herceptin molecule (Transtuzumab) has been shown to demonstrate a high specificity and affinity for the HER2 receptor and also act as a biologic targeted therapeutic agent against HER2 receptors.

Immunohistochemistry (IHC) for the assessment of the level of HER2 protein expression at the tumor cell membrane.

To check for HER2 gene over expression we do immunohistochemistry.
The results can be either 0 , 1+ , 2 + , 3+ .

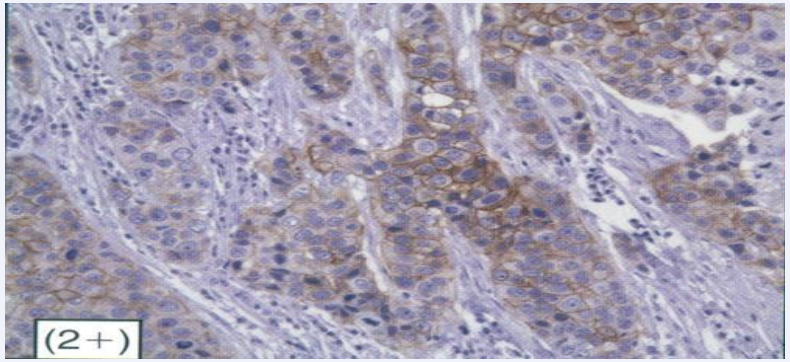


(0) Negative: This one doesn't benefit from Transtuzumab.

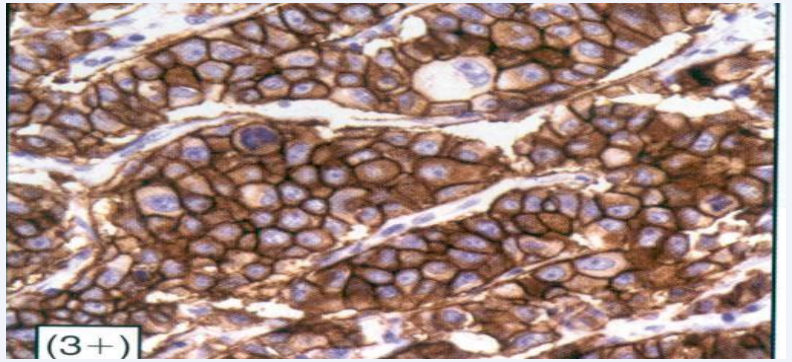


(1+) Also negative : very faint cytoplasmic stain.

Important.



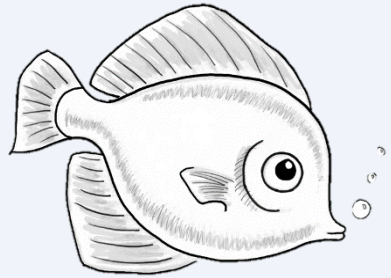
(2+) Either positive or negative for HER2 (Histopathology study can not tell the different), so we have to use another method in this case which is FISH.



(3+) This one is positive and we can give her Transtuzumab.

FISH

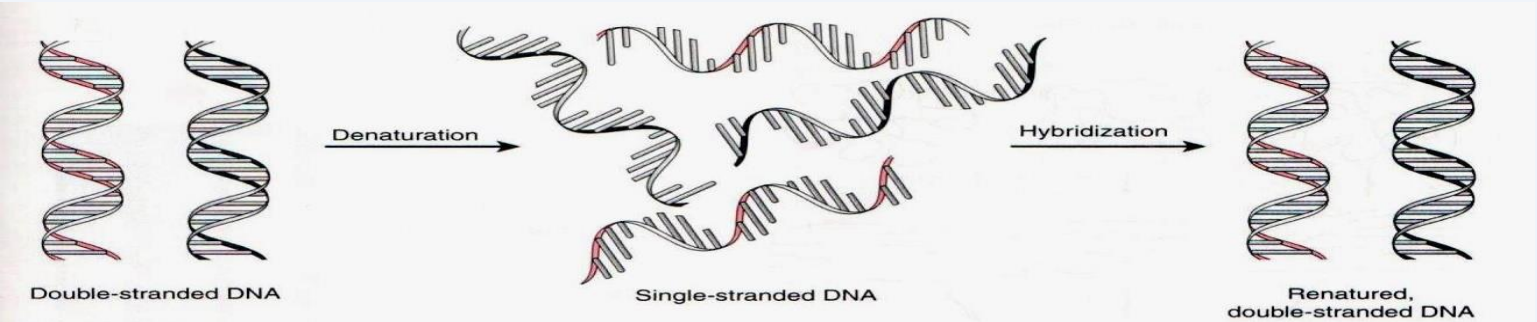
FISH (Fluorescent in situ hybridization)



- We do it for **patients with the result (2+)** after the imaging with immunohistochemistry for HER2.

How to do the FISH ? (اقرؤوها عشان تفهمون السلايد اللي بعدها)

- First return to the structure of the DNA, the DNA is a double helix composed of a sequence of amino acids, held together by bonds.
- The first step we do is the Denaturation of DNA , we do that by heat or alkaline to break the bonds between the DNA, so we will have a single strand instead of the double stranded DNA.
- Then we will bring a hybrid strand that is labeled with flurochrome, and it will be hybridized with the single strand that we have.
- This process will show us how many copies of the HER2 gene is expressed.



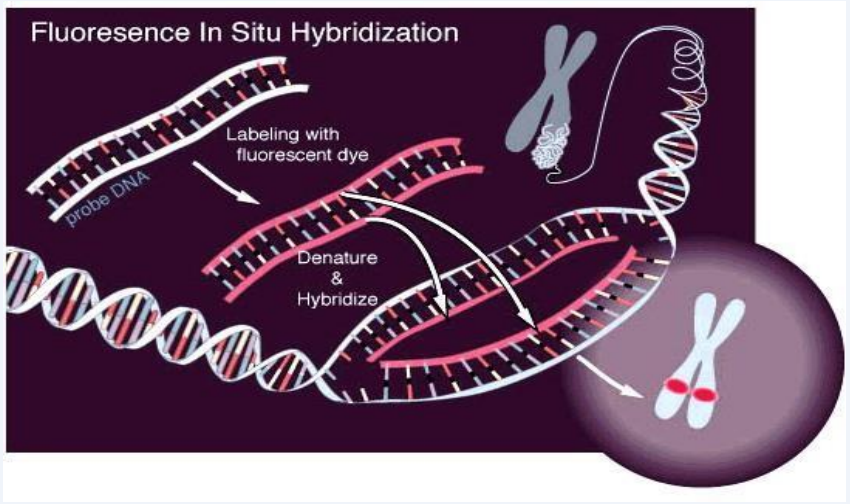
The principle of nucleic acid hybridization. The two complementary strands of a Watson-Crick double helix can be "denatured" by a variety of treatments (such as high temperature, high pH, or very low salt conditions) to yield a collection of single-stranded DNA molecules. Under conditions that favor formation of renatured double-stranded DNA, complementary strands will "hybridize" to each other, but not to other fragments of DNA that have a different nucleotide sequence.

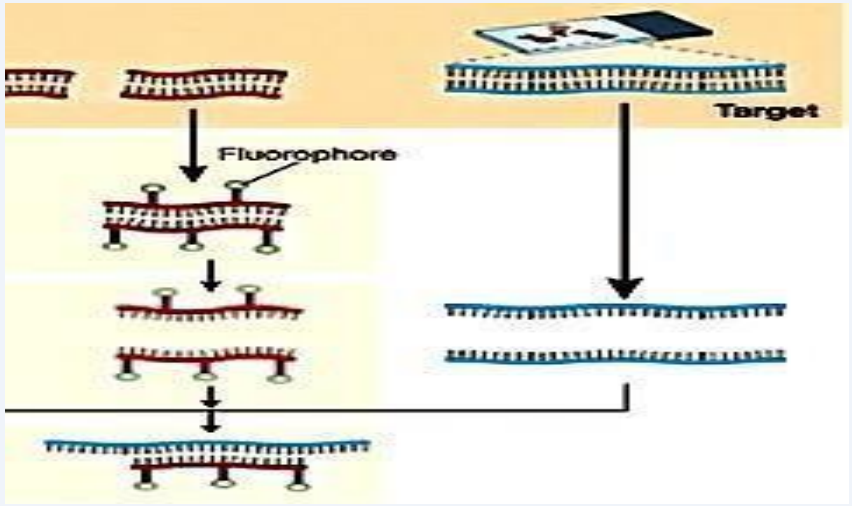
(If you are interested in Genetics then go ahead and study it, otherwise it is just an extra reading)

FISH

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.





Classification with the treatment

This is extremely IMPORTANT

According to all the mutations before, we classified the breast cancer into:

- Each one of the patients with a breast cancer has to be under one of these.

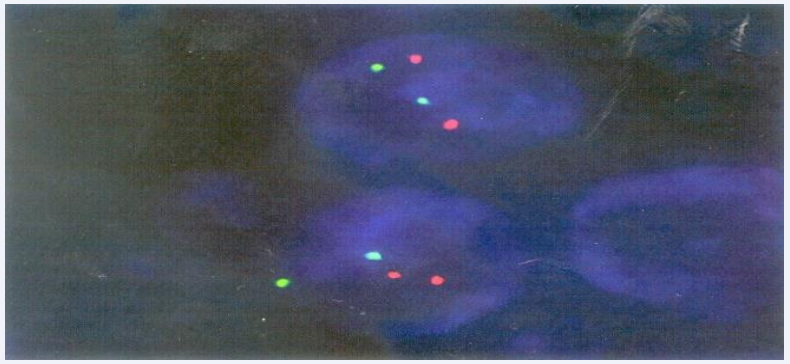
Immunophenotyping as a Surrogate for molecular category using Estrogen receptor, progesterone receptor and HER2 Status				
Molecular Category				
	Luminal A	Luminal B	HER2	Basal-Like
ER (Estrogen receptor)	+	+	-	-
PR (Progesterone receptor)	+	+	-	-
HER2	-	+	+	-
Treated with	Tamoxiphen	Tamoxiphen and Transtuzumab	Transtuzumab	Chemotherapy (Triple negative)

- Number one bad mutation is HER2 and then Triple negative.
- Being negative is not meant to sound good, it means we don't know where is the mutation so treatment will be empirical (Chemotherapy).

(Don't worry about it)

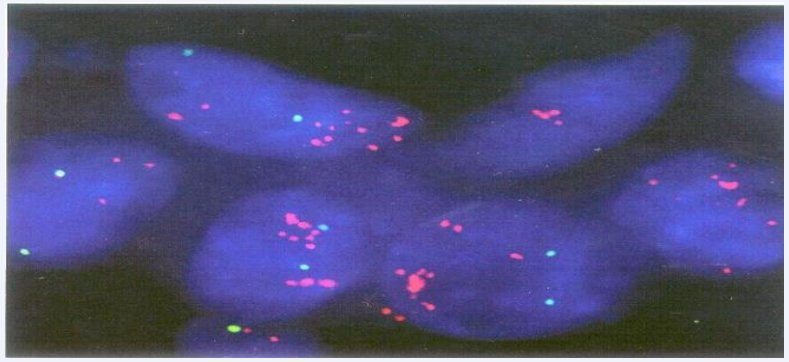
FISH and SISH

Florescence in Situ Hybridization images



This one is negative

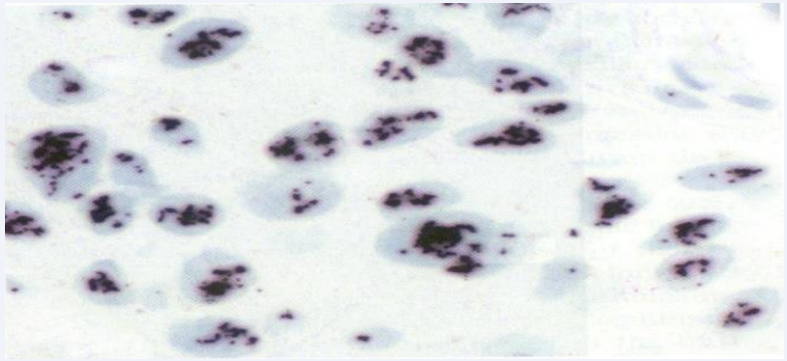
- Chromosome 17 is the green dot.
- HER2 is the red dot.



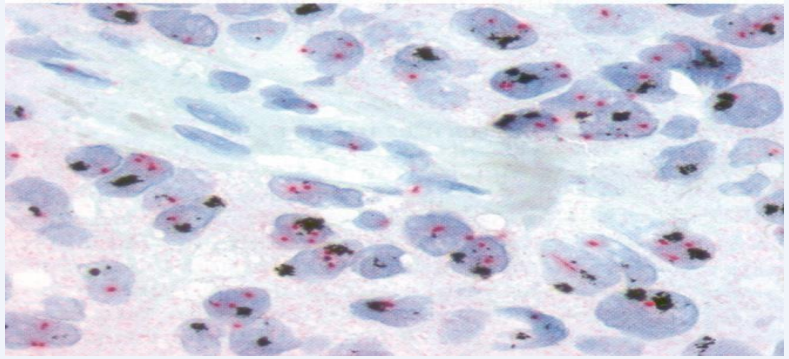
This one is Positive

- Many copies of HER2 (red dots).

SISH and Dual color silver in situ hybridization



SISH uses **Silver** instead of Florescence

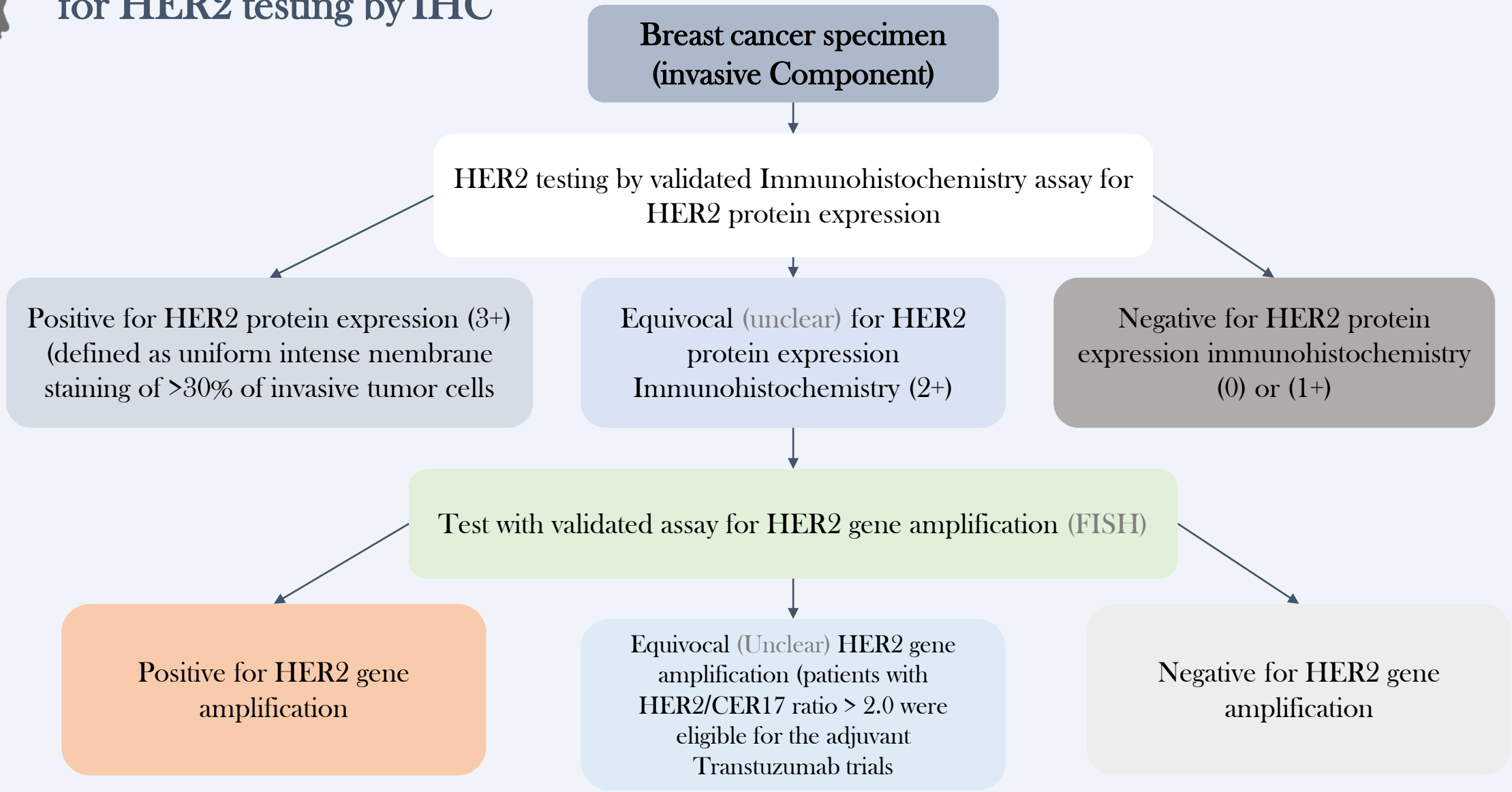


Dual Color

Important

Summary for HER2 mutation

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



SUMMARY

BRCA1 and BRCA2 are mutated in familial breast cancer.

- BRCAI is located on chromosome 17q.
- BRCA2 is located on chromosome 13q.

Most types of breast cancers are hormone dependent : estrogen (ER) , progesterone (PR)

- HER2 positive breast cancers tend to be aggressive.
- Normal cells have one copy of the HER 2 gene on each chromosome 17 (CHR17).

Treatment

- ER : tamoxifen or aromatase inhibitor therapy .
- HER2: Herceptin molecule (Trastuzumab) .

- Diagnostics for genetic mutations in Brest cancer:

1/ Immunohistochemistry (IHC) ,for knowing level of HER2 protein expression.

2/ Fluorescent in situ hybridization (FISH) , for patients with (2+) in IHC.

3/ Silver in situ hybridization (SISH).

- Steps of Investigating Breast cancer :

1/Screening (mammogram).

2/ biopsy (grading).

3/ IHC.

Molecular Category

Gene	Luminal A	Luminal B	HER2	Basal-Like
ER	+	+	-	-
PR	+	+	-	-
HER2	-	+	+	-
Treated with	Tamoxifen	Tamoxifen & Trastuzumab	Trastuzumab	Chemotherapy

Test Yourself!!

Q1. which one of the following is FALSE regarding BRCA 1 gene?

A. It is a DNA repair gene
B. It is located on the chromosome 17q
C. It is only involved in breast cancer
D. Mutation in it is more aggressive than BRCA 2

Q2. A 39 years female presented for a routine check up, on examination a mass was found in her right breast, a biopsy was taken from the mass and ERs was seen ,some degree of mitosis in the ductal cells. Which one of the following is the best to be used for the treatment?

A. Transtuzumab
B. Aromatase inhibitor Thereby
C. Tamoxiphen
D. B&C

Q3. Immunohistochemistry for HER2 gene from a breast specimen was found to be (2+). What should be the next step?

A. Start trantuzumab
B. Chemotherapy
C. Use FISH method
D. Start Tamoxiphen

Q4. How many copies of HER2-neu gene is normally expressed on cells?

A. One copy
B. Two copies
C. Five copies
D. 20 Copies

Q5. Normal function of HER2-neu gene is ?

A. Tumor suppressor gene
B. DNA Repair gene
C. Sex determination gene
D. Regulates cell growth by protein production

Q6. BRCA2 gene is located in which chromosome?

A. Chromosome 11p
B. Chromosome 17q
C. Chromosome 13q
D. Chromosome 13p

Answer key:

1:C 2:D 3:C 4:B 5:D 6:C

"One page can change you, many will change the world"

Team Leaders:

Mohammed Habib. **Aseel Badukhon.**

Special thanks to:

Nada Al-Somali.

"تم بفضل الله ♥

آخر محاضرة لمقرر الوراثة البشرية في مسيرة دراستنا في كلية الطب و كل ما نتمناه هو أن يكون هذا العمل في ميزان حسناتنا و علم ينتفع به بإذن الله و دعواتنا لكم بالتوفيق و النجاح"

Reference

Male's and Female's Slides