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**DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE**

**Corso di Laurea**

**Scienze Biologiche**

**“LA SOTTOREGOLAZIONE DI CYB5DL È  
ASSOCIATA ALLA PROGRESSIONE DEL CARCINOMA  
MAMMARIO”**

**“DOWNREGULATION OF CYB5DL IS ASSOCIATED WITH  
BREAST CANCER PROGRESSION”**

Tesi di Laurea di:  
di:  
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# Introduction

It is already known that Breast cancer or "BC" is induced by the mutations of several genes, the so-called "tumor suppressors", as TP53 or GATA3.

CYB5D2 (Cythochrome b5 domain containing 2), a MAPR protein coding gene located at 17p13.2 is considered a new member of this group after the discovery that it is downregulated in this type of cancer.

Studies made over 3000 primary BCs suggest that this gene is implied in the inhibition of cancerous cells multiplication.

# Methods and materials

Researchers with the purpose to understand the role of CYB5D2 in BC tumorigenesis have used the MCF7 cell line to obtain TAM-R cells (tamoxifen resistant cells). Results have been reported then in cancer genomic programs like TCGA or Curtis data sets.

Studies have been carried out following these passages (image 1):

- I. Tissue culture and the **development of tamoxifen resistant cells;**
- II. TUNEL apoptotic detection;
- III. Knockdown of CYB5D2 and proliferation assay;
- IV. Western blot;
- V. Determination of TAM-derived cytotoxicity;
- VI. Treatment of xenograft tumor with TAM,
- VII. Immunohistochemistry (IHC) analysis of CYB5D2 expression;
- VIII. ER-promoter assay;
- IX. Real time PCR analysis
- X. Pathway enrichment analysis;
- XI. Statistical studies.

# Results

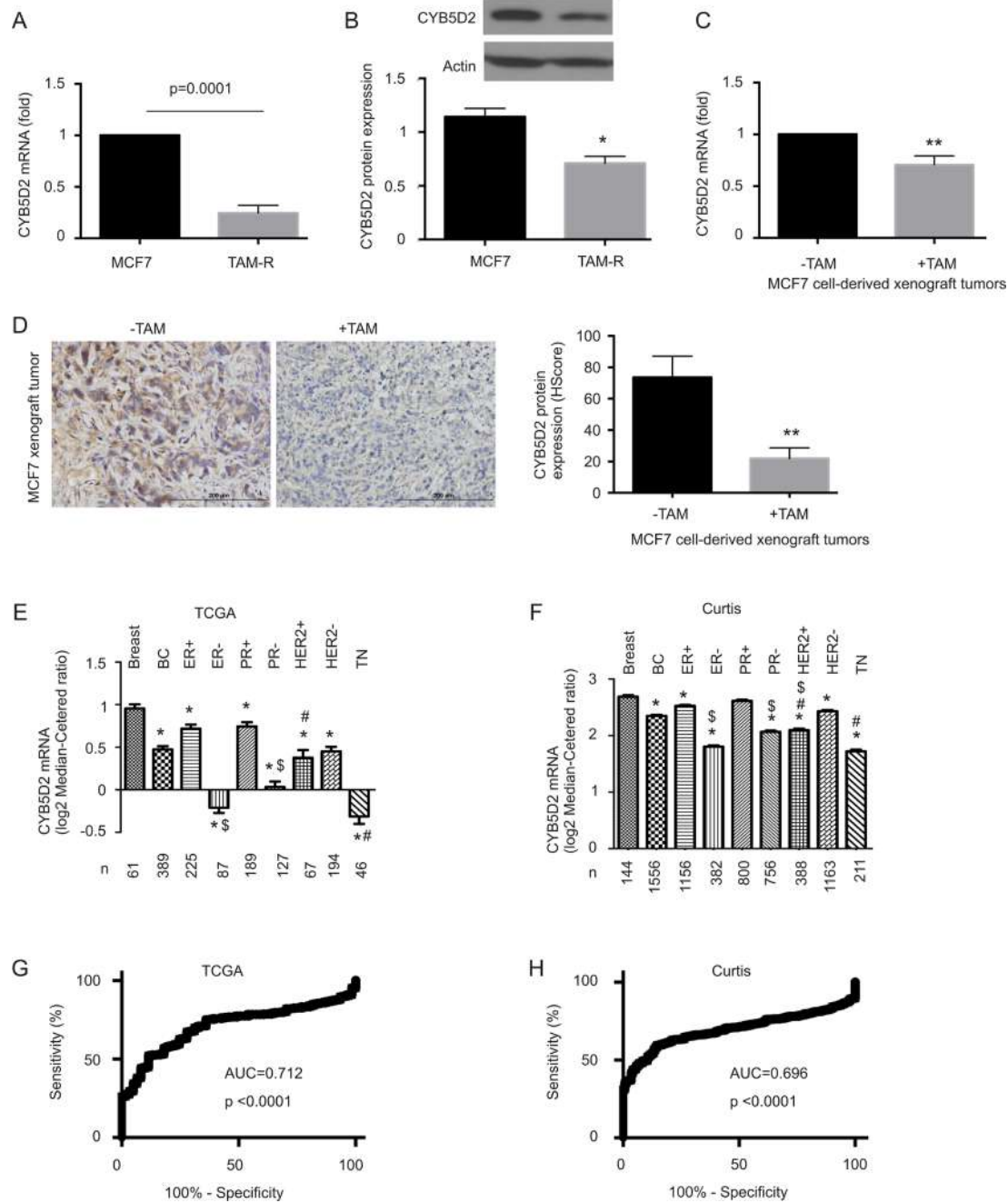


Image 1

- Downregulation of CYB5D2 has been detected both in TAM-R and xenograft cancer cells, while the transfection of MCF7 cells with GFP and a GFP-CYB5D2 fusion protein has confirmed the fact that CYB5D2 **causes apoptosis in cancerous cells**: in fact, GFP cells were more numerous than GFP-CYB5D2 ones (image 2, figures A and B).

- It has been discovered that there are mutated genes related to CYB5D2 downregulation, which are grouped into "Signature #1" and "Signature #2": the first is made up by altered tumor suppressors as TP53, while the second is composed by 21 DEGs (selected from Metabric dataset). They both **bring to a reduction of overall survival in BC and its subtypes** (image 3, figure B and C for S#1, image 4 for S#2)

# Results

- It has been found out that pathways regulating cell cycle, oocyte maturation, checkpoints activation or p53 transcription are enriched in DEGs related to CYB52 reduced expression.

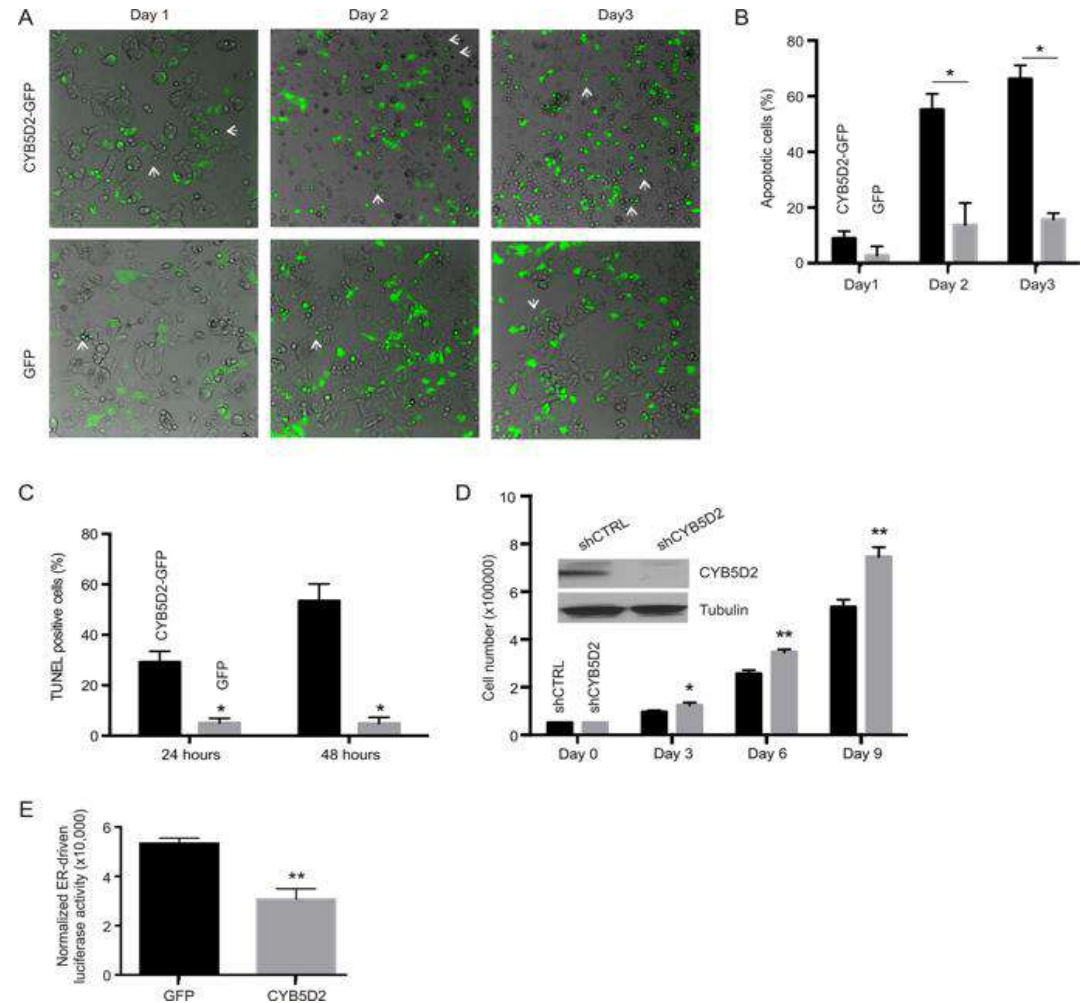


Image 2

# Discussion

CYB5D2 is a tumor suppressor whose mutation causes the development of breast and cervical cancers, characterized by the deletion of its chromosomal localization. Its downregulation **is associated with the inactivation of p53 and other oncogenic factors, as GATA3(Signature# 1)**. CYB5D2 is capable of stopping MCF7 cells proliferation, while its reduction is also related with 21 differentiated expressed genes named "Signature#2", which are responsible with Signature# 1 for the Overall survival diminution in BCs. There are some pathways involved in mitotic events which are affected by DEGs correlated with CYB5D2 reduction: this confirms that this gene **regulates cells duplication.**

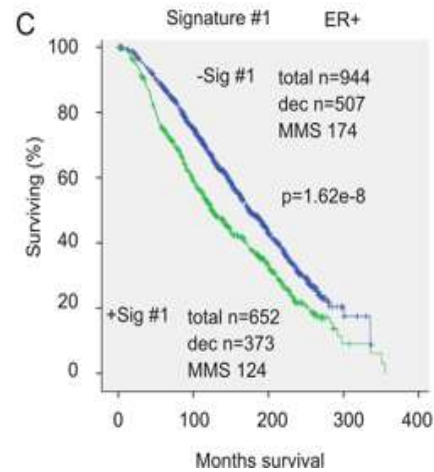
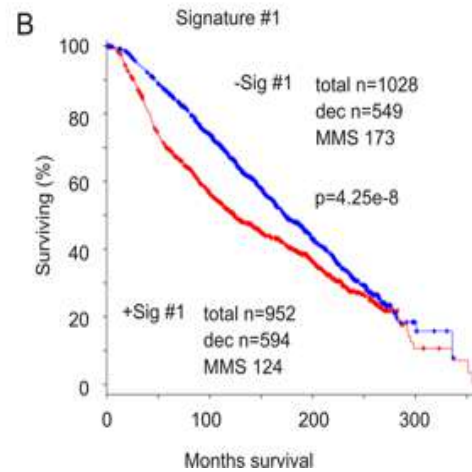
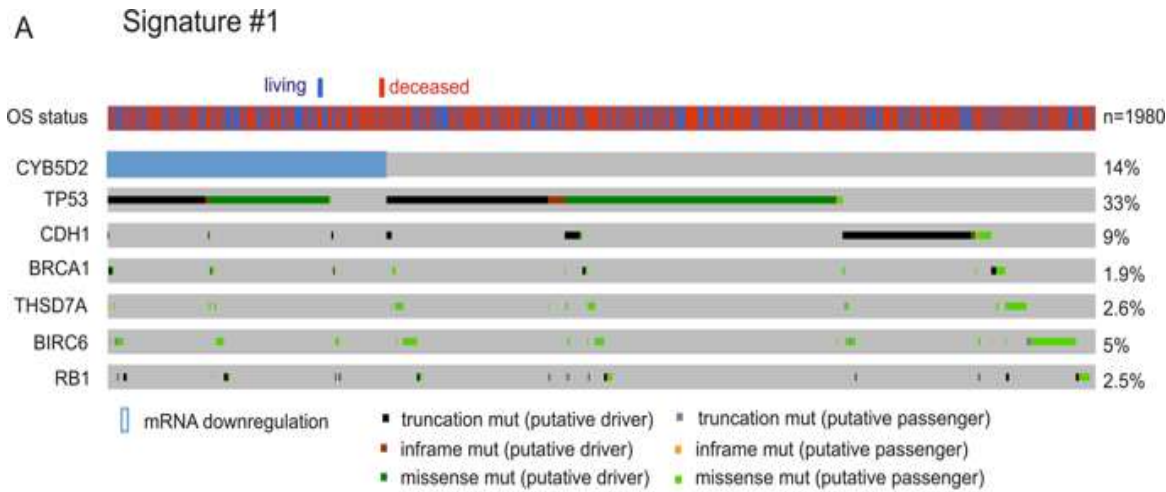


Image 3

# Conclusion

A new breast cancer suppressor has been discovered: it is CYBD2. Its mechanisms are still unclear, but now researchers have another piece to complete the puzzle of this cancer oncogenesis.

The hope is to find the answers for those questions made years ago, when there was not way of salvation for BC patients.

Scientific research is shy, but it is strong and will never stop its fight against cancer.

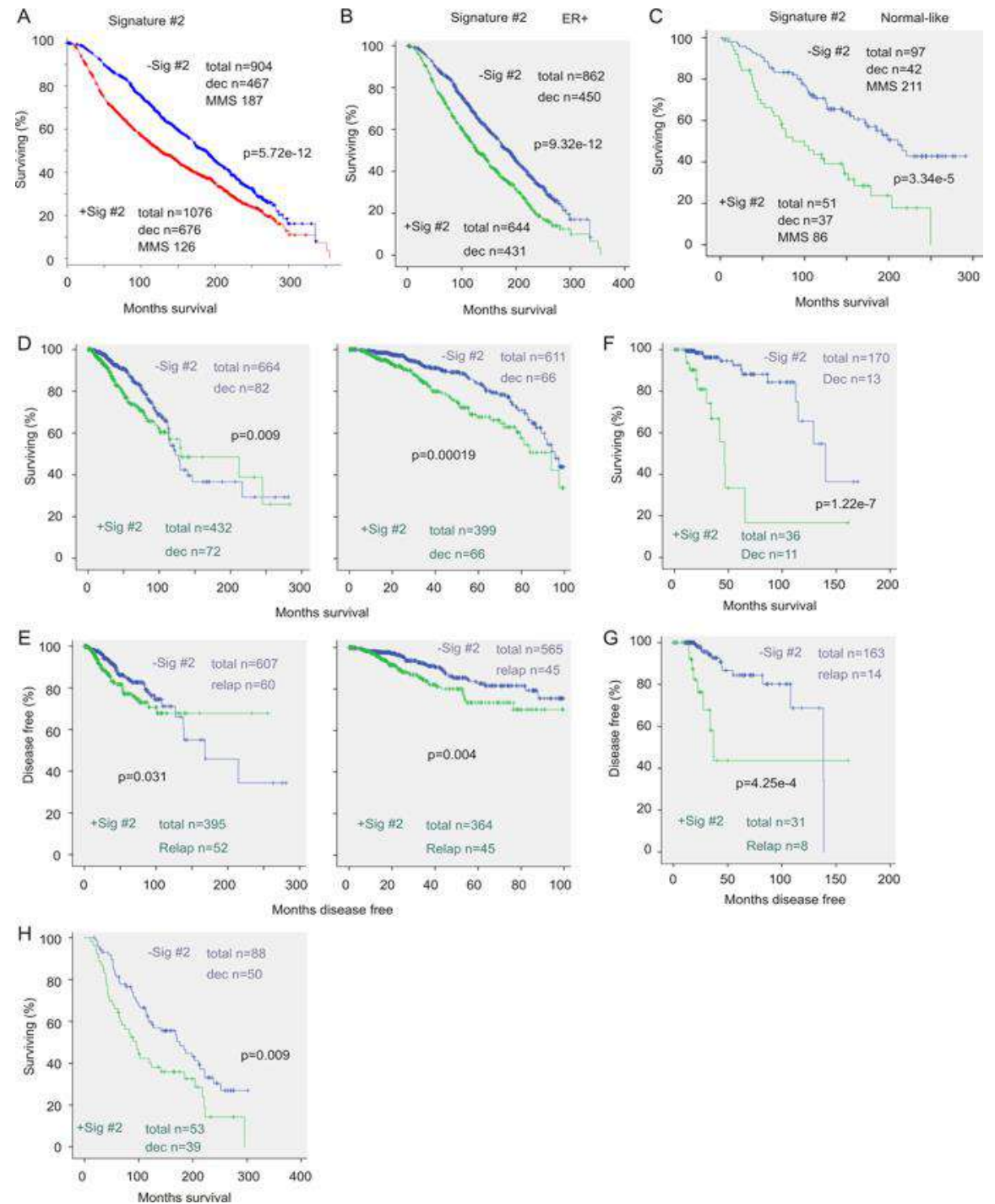


Image 4

# Bibliography

- "genetica–un approccio molecolare" by Peter J.Russel, "Pearson" publishing house
- "Downregulation of CYB5D2 is associated with breast cancer progression",an article written by Djane Ojo,David Rodriguez,Feuxiang Wie,Anita Banc and Damu Tiang,published on "Nature" in april **2019**.



## ***La diminuzione dell' espressione del gene CYB5D2 è associata alla progressione del carcinoma mammario***

CYB5D2 (“dominio del citocromo b5 contenente 2”) è un oncosoppressore di recente scoperta la cui espressione ridotta è riscontrata sia nel carcinoma mammario che in quello cervicale. Situato nella regione 17p13.2, è un gene codificante per una proteina della famiglia MAPR la quale, analogamente a quanto avviene nella promozione del differenziamento dei neuroni, **inibisce la replicazione delle cellule cancerose**. Uno studio condotto su oltre 3000 tumori al seno primari con l'impiego di cellule MCF7, finalizzato ad ottenere cellule resistenti al tamoxifene tramite vari passaggi che comprendono anche una analisi PCR in tempo reale, ha confermato la responsabilità di CYB5D2 nell' oncogenesi del cancro alla mammella. Gli oncosoppressori come GATA3 ed i **21 geni espressi in modo differenziato** mutati ed associati alla ridotta espressione di CYB5D2 sono conosciuti rispettivamente come **“Firma#1”** e **“Firma#2”** e riducono sia **la sopravvivenza globale** che **la sopravvivenza libera dalla malattia** degli individui affetti da carcinoma mammario. La scoperta inoltre che alcuni **pathways** deputati alla regolazione del ciclo cellulare, all'attivazione dei checkpoints o alla trascrizione di p53 presentano dei **DEGs correlati alla riduzione dell' espressione di CYB5D2** confermano ulteriormente il suo essere un oncosoppressore; un altro tassello è stato aggiunto al puzzle del tumore al seno.

N.B.: sono stati consultati i datasets di programmi come Curtis o Metabric.