TUMOR SUPPRESSORS AND ITS ROLE IN CANCER

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Abstract: Cancer can start from any part of the body which is made up of 3 trillion cells. Cancer is caused due to an abnormality in genes that leads to proliferated, undifferentiated mass of cells. There are mutations in sequences called drivers of cancer. In oncology, the drivers of cancer are the changes in proto-oncogenes, tumor suppressor genes and DNA repair genes. Belonging to this category are the 9 genes P53, APC, HER 2, CDK 4, BRCA1, BRCA2, PTEN, TP53 & RB1. In our work, we have considered oncogenes, tumor suppressor genes and have done domain analysis. In domain analysis we have done comparisons among all the cancer genes, checked for the similarities and also done sequence analysis. These analysis could help in predicting the relation and their involvement in cancer.

Keywords: Tumor Suppressor Genes, Prosite.

Introduction: Genes are found in every cell that makes up the body. Genes control the way, the cells work by making proteins that have specific functions. All cancers begin when one or more genes in a cell are mutated, or changed. This creates an abnormal protein or no protein at all. An abnormal protein in the cell division machinery provides different information than a normal protein, which can cause cells to multiply uncontrollably and become cancerous.

There are two basic types of genetic mutations:

- **Acquired mutations** are the most common cause of cancer. These occur from damage to genes during a person's life, and they are not passed from parent to child. Factors such as tobacco, ultraviolet (UV) radiation, viruses, and age cause these mutations.
- **Germ line mutations**, are less common, and are passed directly from a parent to a child. In these situations, the mutation can be found in every cell of a person's body, including the reproductive sperm cells in a boy's body and egg cells in a girl's body. Thus, it passes the mutations from generation to generation. Cancer caused by germ line mutations is called inherited cancer, and it makes up about 5% to 10% of all cancers.

Literature Review: Mutations happen often, and the human body is normally able to correct most of them. Depending on where in the gene the change occurs, the mutation may be beneficial, harmful, or make no difference at all. So, one mutation alone is unlikely to lead to cancer, it takes multiple mutations over a lifetime to cause cancer. Cancer is defined as a disease in which there is an abnormal, uncontrollable cell division disregarding the rules of normal cell-division. If the proliferation is allowed to continue it can be fatal- accounting to 90% of cancer related deaths.(22)

Initiation and progression of cancer depends on both external and internal factors. The external factors include the environmental, chemical, radiation and the internal ones could be because of inherited mutations, hormones etc. These factors act together resulting in the abnormal cell behavior resulting in proliferation of cells. Spreading of excessively proliferating cells is metastasis, DNA mutations in the cell signaling machinery converts the normal cell into a cancerous cells.

A model proposed by Douglas Hanahan and Robert Weinberg which explains the possible causes of the formation of tumors.

1. Excessive cell divisions because of any physical or chemical factor may eventually form a mass called a **tumor**
2. Continuous cell divisions and limitless replication i.e. **immortality**
3. Resistance to self-death- **apoptosis**.
4. Spread of cancerous cells to other sites- **metastasis**.
5. Long term production of **Go signals** i.e. growth factors of oncogenes.
6. Deactivation of stop signals- in turn off of tumor suppressor genes.

Tumor suppressor genes are protective genes, which suppresses the formation of tumor. Normally, they limit cell growth by monitoring how quickly cells divide into new cells. When a tumor suppressor gene is mutated, cells grow uncontrollably.

Oncogenes turn a healthy cell into a cancerous cell. *HER2*, which is a specialized protein controls cancer growth and spread cancer cells, these are found on breast and ovarian cancer cells(23).DNA repair genes fix mistakes made when DNA is copied. But if a person has an error in a DNA repair gene, these mistakes are not corrected, and they then become mutations, eventually leading to a cancer as seen in lynch syndrome.

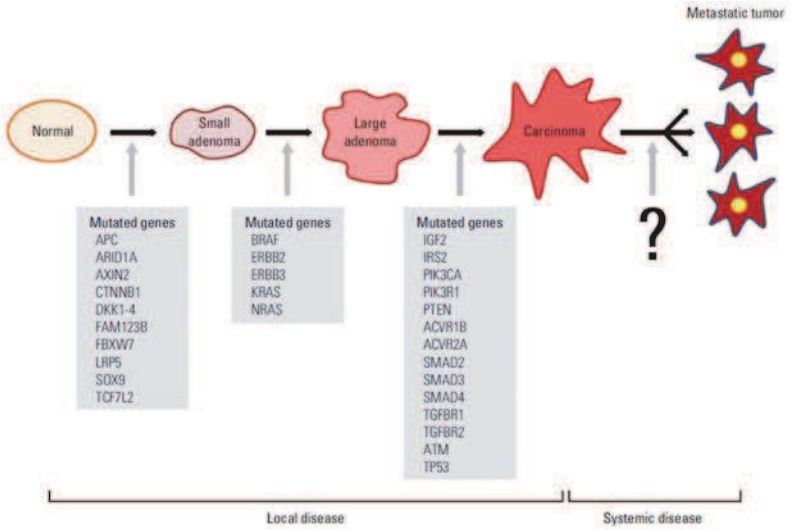

Fig 1: Mutations in Genes Resulting in Cancer

Table 1: Functions of Genes

Gene	Location	Acts as	Functions in
APC Adenomatous polyposis coli	5q22.2	Tumor Suppressor	Plays a critical role in several cellular processes and keeps cells from growing and dividing too fast or in an uncontrolled way.
HER2 Human Epidermal growth factor Receptor 2	17q12	oncogene	HER2 gene amplification results in overexpression, making too many HER2 receptors, thereby makes breast cells grow and divide in an uncontrolled way
CDK4 Cyclin-dependent kinase-4	12q14.1	Tumor Suppressor	Is a protein-serine kinase involved in the cell cycle, responsible for the phosphorylation of RB gene product
BRCA1, BRCA2 *BReast CAncer gene*	17q21.31, 13q13.1	Tumor Suppressor	functions to repair cell damage and keep breast, ovarian cells growing normally, and also plays an essential role in embryonic development.

RB1 Retinoblastoma-associated protein	13q14.2	Tumor Suppressor	mutations in the RB1 gene have resulted in retinoblastoma, a rare type of eye cancer.
TP53 Tumor Protein P53	17p13.1	Tumor Suppressor	p53 activates genes to fix the DNA damage and if the DNA cannot be repaired, this protein prevents the cell from dividing and signals it to undergo apoptosis
PTEN Phosphatase and TENsin homolog	10q23.31	Tumor Suppressor	This enzyme is a part of a chemical pathway that signals cells to stop dividing and triggers cells to self-destruct through a process called apoptosis.

Methodology: PROSITE is a method of determining what is the function of uncharacterized proteins translated from genomic or cDNA sequences. It consists of a database of biologically significant sites and patterns formulated in such a way that with appropriate computational tools it can rapidly and reliably identify which known family of protein (if any) a new sequence belongs to.

In some cases the sequence of an unknown protein is too distantly related to any protein of known structure to detect its resemblance by overall sequence alignment, but it can be identified by the occurrence in its sequence of a particular cluster of residue types which is variously known as a **pattern, motif, signature, or fingerprint.** These motifs arise because of particular requirements on the structure of a specific region(s) of a protein which may be important, for example, for their binding properties or for their enzymatic activity. The use of protein sequence patterns (or motifs) to determine the function(s) of proteins is becoming very rapidly one of the essential tools of sequence analysis. This reality has been recognized by many authors, as it can be illustrated from the following citations from two of the most well known experts of protein sequence analysis, R.F. Doolittle and A.M. Lesk:
"There are many short sequences that are often (but not always) diagnostics of certain binding properties or active sites. These can be set into a small subcollection and searched against your sequence".

"In some cases, the structure and function of an unknown protein which is too distantly related to any protein of known structure to detect its affinity by overall sequence alignment may be identified by its possession of a particular cluster of residues types classified as a motifs. The motifs, or templates, or fingerprints, arise because of particular requirements of binding sites that impose very tight constraint on the evolution of portions of a protein sequence (2)."

PROSITE is a database of protein families and domains. It is based on the observation that, while there is a huge number of different proteins, most of them can be grouped, on the basis of similarities in their sequences. Proteins or protein domains belonging to a particular family generally share functional attributes and are derived from a common ancestor.

PROSITE currently contains patterns and profiles specific for more than a thousand protein families or domains. Each of these signatures comes with documentation providing background information on the structure and function of these proteins.

InterPro provides functional analysis of proteins by classifying them into families and predicting domains and important sites. To classify proteins in this way, InterPro uses predictive models, known as signatures, provided by several different databases (referred to as member databases) that make up the InterPro consortium. We combine protein signatures from these member databases into a single searchable resource, capitalising on their individual strengths to produce a powerful integrated database and diagnostic tool.

Based on the lengths of the genes the domain sizes and number varied, and by the observations it was found that the domain number remained constant for most of the genes. Any mutations in the above

said domains result in functional changes leading to alterations in the functionality of the genes. Few of the alteration are

Results and Discussion: Domains were identified by the tool Prosite available at EXPASY. By this tool we found the number and also the location of the domains for each gene.

Table 1: Domain Information

S.no	Gene	Size (Amino acid residues)	No. of Domains	Locations in the gene	Interpro Ids	Domains
1.	**APC**	619	3	660-702 1189-1421 2239-2581	IPR032038 IPR009234 IPR009232	ARM_REPEAT SERINE RICH
2.	**BRCAI**	396	4	24-65 345-507 1642-1736 1756-1855	IPR001841 IPR0018957 IPR025994 IPR025994	Zinc finger domain Serine rich domain BRCT domain BRCT domain
3.	**BRCA2**	483	4	2482-2667 2670-2794 2831-2872 3052-3185	IPR015252 IPR015187 IPR015205 IPR015188	Brc-2 Susceptibility prtn BRCA2, OB1 Tower domain BRCA2,OB3
4.	**Rb**	604	4	103-229 373-573 646-765 768-927	IPR024599 IPR002720 IPR002719 IPR015030	Rb associated prtn N-terminal Rb associated prtn A-box Rb associated prtn B-box Rb associated prtn C-terminal
5.	**CdK4**	289	1	6-295	IPR000719	Protein kinase domain
6.	**Her2**	790	5	52-172 190-343 366-485 511-642 720-987	IPR000494 IPR006211 IPR000494 IPR032778 IPR000719 IPR001245 IPR020635	Receptor L-domain Furin like cysteine rich domain Receptor L-domain Growth factor domain4 Prtn kinase Ser-thr kinase-catalytic Tyrosine kinase-catalytic
7.	**P53**	250	3	6-30 100-288 319-357	IPR013872 IPR011615 IPR010991	P53 transactivation domain P53 DNA binding domain P53 tetramerization domain
8.	**pTen**	391	4	14-185 23-183 101-159 188-350	IPR029023 IPR014020 IPR003595 IPR000340	Tensin type phosphatase Tensin phosphatase c2 Tyrosine phosphatase catalytic Dual specificity phosphatase and catalytic domain

1. **APC**- APC functions in few cellular processes such as in cell migration, adhesion and in chromosomal segregation. APC functions as a tumor suppressor and any mutation in the MCR regions leads to loss of the suppressing activity, like loss of domains required for binding to microtubules, promoting tumorigenesis, and loss of adhesion(1,2).

2. **BRCA1**, **2**- these are tissue specific tumor suppressor genes. These genes function to help in repairing the damaged DNA thus plays a role in ensuring the stability of cells genetic material. If these genes are mutated the alterations mainly effects breast and ovaries leading to Breast ovarian cancer syndrome. (3,4,5)
3. **Rb protein**- is a tumor suppressor, the transition of cells from G1 to S phase is under the control of Rb-E2F which gets activated after Rb interacting with cdk4. Any deregulation of Rb-E2F results in cancer. (18,19, 20)
4. **Cdk 4**- functions as a tumor suppressor, controlling the progression of cells through G1 phase of the cell cycle. The activity of cdk4 is inhibited by p16 protein, after its inhibition it no longer controls the progression through G1 phase, thereby results in cell proliferation.(6,7)
5. **Her2**- functions as a tumor suppressor, normally the gene codes for a protein present on the surface of cells and a healthy breast cell has two copies of the HER2 gene. Overexpression of her-2 was found in several cases of breast cancer. Over-expression of her-2 resulted in the dysregulation of EGF receptor signaling, which results in greater cell proliferation and tumor promoting activities- tumor proliferation was found to be high in s-phase cells. Human breast carcinomas have been found upon over-expression.(8,9,10)
6. **P53**- is a tumor suppressor, whose levels are normally low, any variation in the levels. Triggers stress conditions, resulting in either repairing the genes or prompting for the cell arrest leading to excessive apoptosis.(11,21)
7. **PTEN**- is a tumor suppressor, its role is intended to be homeostatic maintenance of P13K- AKT cascade, whose role is to activate a cascade of proteins, which help in maintaining the lipid secondary messenger (PIP), PTEN was found to be either mutated or lost in cancer patients.(12,13,14,15,16,17)

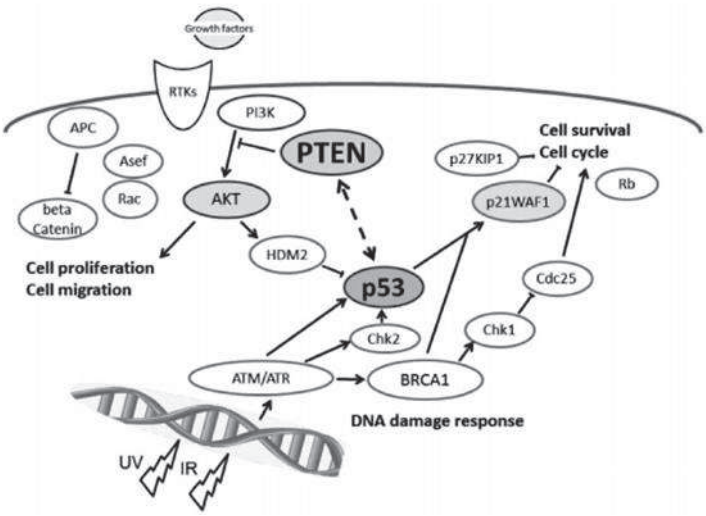

Fig 2: Mechanism after DNA Damage

Conclusions: Our work on nine different genes which play a key role in the development of Cancer were studied by using PROSITE interconnected to Interpro to analyse the domains and motifs on proteins. The analysis showed that many of the genes contain different types of domains, with mostly binding capabilities and mostly have same number of domains.The mutations in any of the domains may cause inactive protein or may lose its specific binding ability which causes Cancer.

Future Works: Further analysis has to be performed using cancer patients genome to find the exact locations on all the genes possible for causing cancer. By doing so we can use several techniques like the RNAi, crispr-cas, zinc-finger domains to block the mutated gene.

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