Tumor suppressor genes

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What is a Tumor Suppressor Gene?

- A *tumor suppressor gene is a type of cancer gene that is created by loss-of function* mutations.
- In contrast to the activating mutations that generate oncogenic alleles from proto-oncogene precursors, tumor suppressor genes, and the proteins they encode, are functionally *inactivated by mutations*.

- Tumor suppressor genes typically control processes fundamental to the maintenance of stable tissue compartments.

- These processes include:

1. The maintenance of genetic integrity
2. The progression of the cell cycle
3. Differentiation
4. Cell–cell interactions
5. Apoptosis

- The first cancer genes to be discovered were oncogenes. For a time it was widely believed that the cancer phenotype resulted primarily from activating mutations that led to gains of function. An early piece of evidence that other types of genetic alteration might also be important in cancer was provided by Henry Harris and his colleagues.
- In a 1969 study, Harris adopted a novel approach to study the genetic factors that were involved in cancer cell phenotypes.

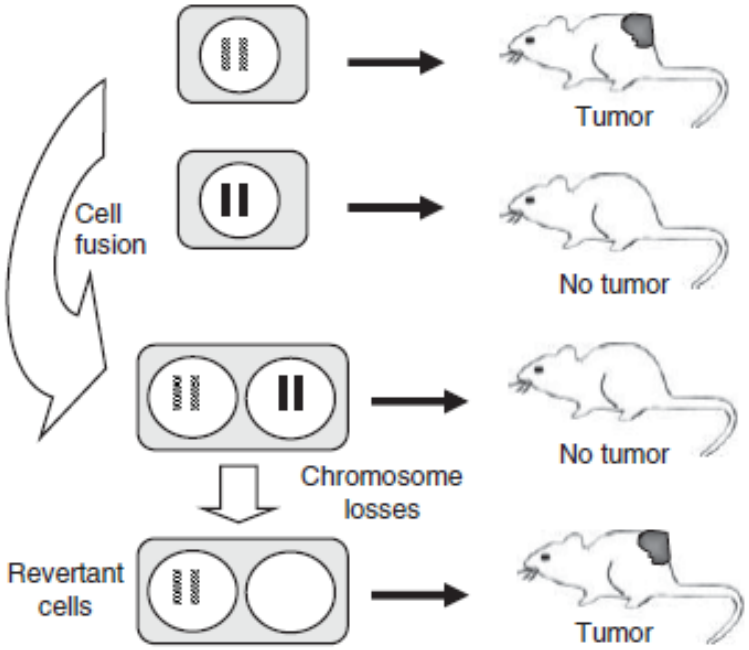


Fig. 3.1 Tumor suppression is a dominant phenotype. Two cell types are isolated from tumors: tumorigenic cells (defined as those that form tumors when introduced into the skin of mice) and non-tumorigenic cells. In this simplified illustration, only the relevant pair of homologous chromosomes is shown in each cell (solid and hatched, respectively). Fusion of these two cells allows them to share their genetic material. Cells containing both sets of chromosomes are not tumorigenic, demonstrating that the alleles that cause tumor formation (carried on the hatched chromosomes) are recessive. Because the chromosome complement of the fused cells are unstable, with time cells appear that have lost wild type alleles (carried on the solid chromosomes) contributed by the non-tumorigenic cells. These rare cells revert to a tumorigenic phenotype

Retinoblastoma and Knudson's Two-Hit Hypothesis

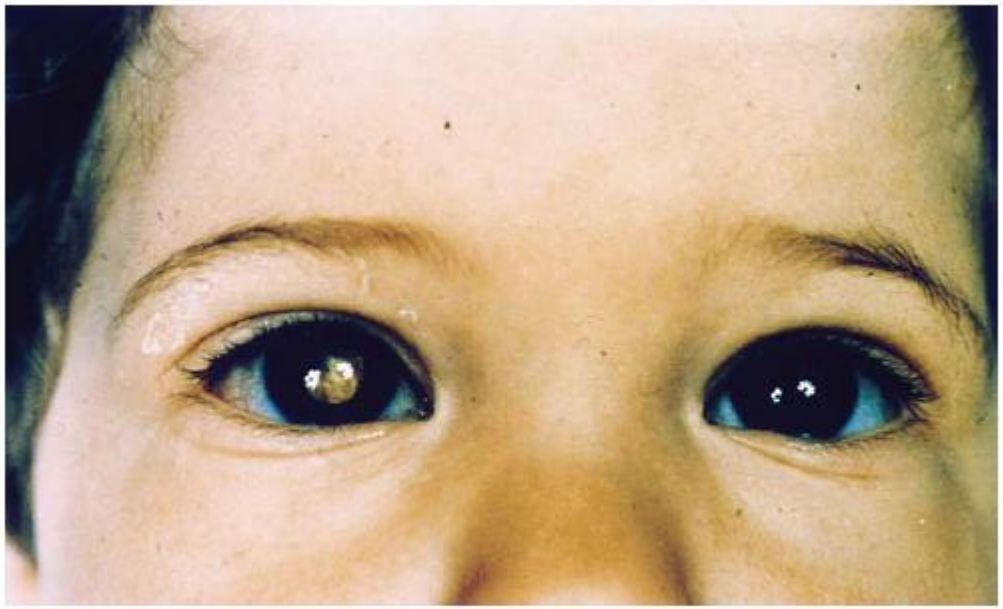


Fig. 3.2 Retinoblastoma. A pediatric malignancy of the retina, retinoblastoma occurs in hereditary and sporadic forms. Shown is a patient with a unilateral tumor (in the eye on the left). (Courtesy of the National Cancer Institute.)

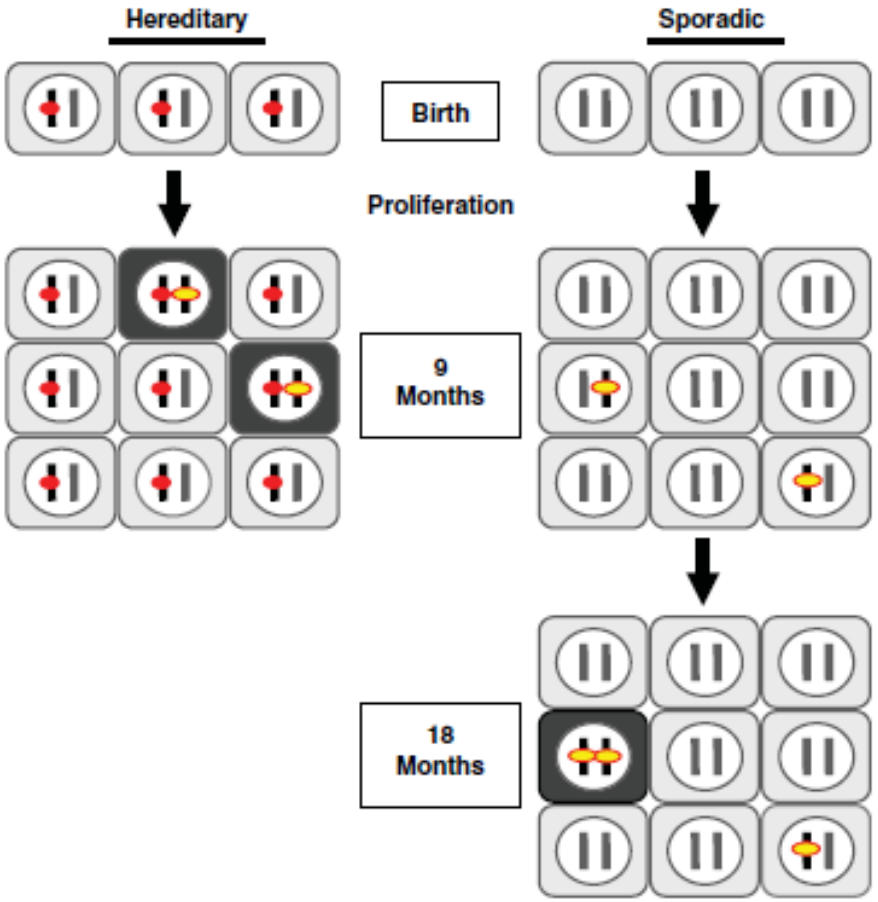


Fig. 3.3 The 'two-hit' hypothesis. At birth, individuals predisposed to the hereditary form of retinoblastoma harbor a mutant allele (shown in red) in every cell, including the blast cells of the retina. These cells proliferate during the first 9 months of life. During this time, somatic mutations at the retinoblastoma locus (shown in yellow) occur at a low frequency. In individuals predisposed to retinoblastoma, the somatic inactivation of a single allele is sufficient to provide the two hits required for tumor formation. Multiple tumor precursor cells (shown as dark cells) are thus generated, leading to bilateral tumors that are often multifocal. In contrast, normal cells require two somatic hits for tumor development – a low probability event. Because of the requirement for two somatic hits, sporadic retinoblastomas are rare and tend to occur at an older age than inherited retinoblastomas

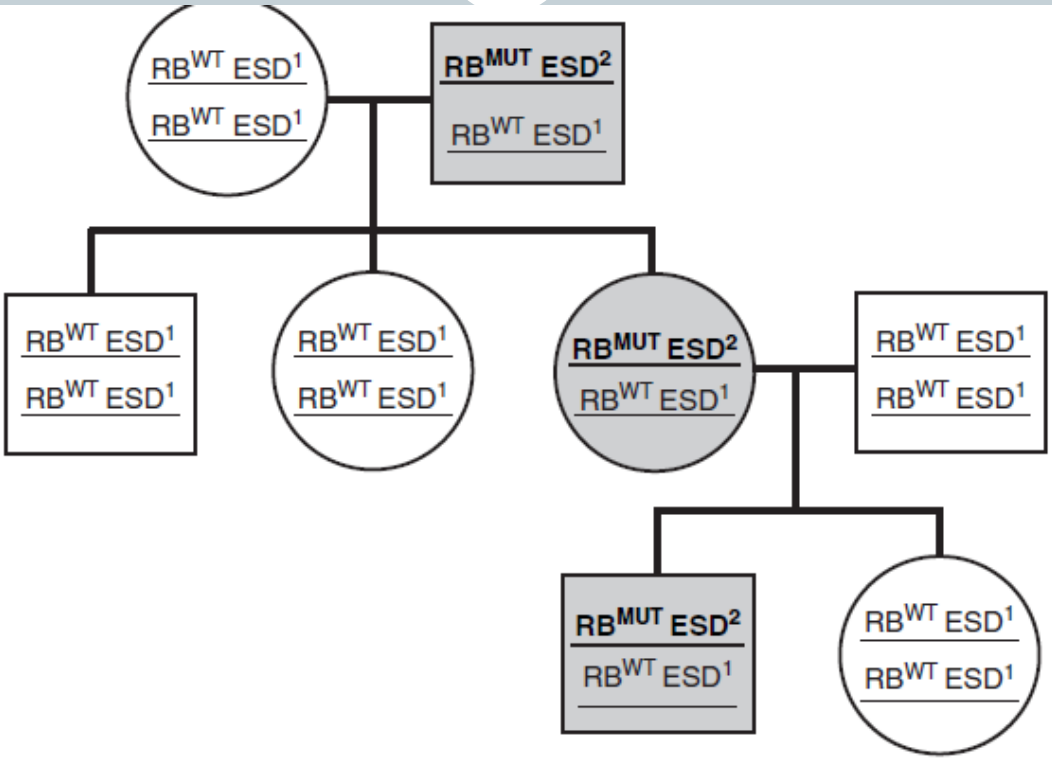


Fig. 3.4 Linkage between the putative *RB* locus and *ESD*. Distinct alleles of the *ESD* gene (denoted ESD^1 and ESD^2), encode proteins that can be resolved by protein electrophoresis. Evaluation of *ESD*-encoded proteins thus provides an assessment of *ESD* allelotype. In retinoblastoma kindreds, allelic variants of *ESD*, when present, invariably cosegregate with disease. In the pedigree shown, circles represent females, squares represent males. Shaded circles and squares represent individuals affected with retinoblastoma

Loss of Heterozygosity

- One of the key observations that guided the discovery of the *RB gene and confirmed* its recessive nature was LOH, the reduction to homozygosity of a locus that previously was heterozygous. LOH is the second 'hit' predicted by Knudson, and represents the loss of the remaining wild type allele of a recessive tumor suppressor gene. With current methods, LOH can readily be assessed by the examination of known single nucleotide polymorphisms (SNPs), which provide convenient and easily detectable genetic markers.

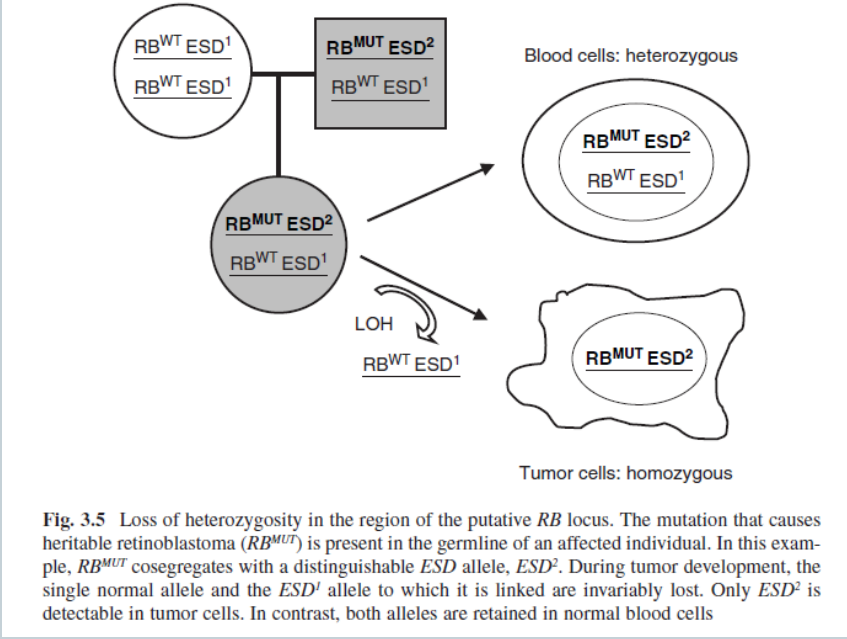
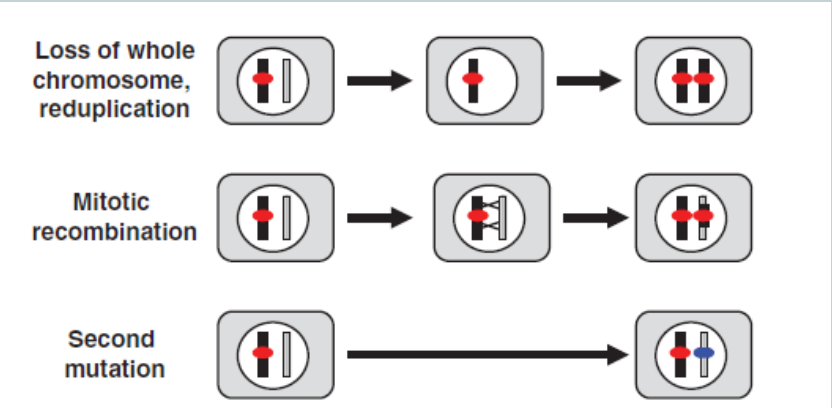


Fig. 3.5 Loss of heterozygosity in the region of the putative *RB* locus. The mutation that causes heritable retinoblastoma (RB^{MUT}) is present in the germline of an affected individual. In this example, RB^{MUT} cosegregates with a distinguishable *ESD* allele, ESD^2. During tumor development, the single normal allele and the ESD^1 allele to which it is linked are invariably lost. Only ESD^2 is detectable in tumor cells. In contrast, both alleles are retained in normal blood cells

Mechanisms of LOH

- *Loss of a whole chromosome*
- *Mitotic recombination*
- *Localized mutations*

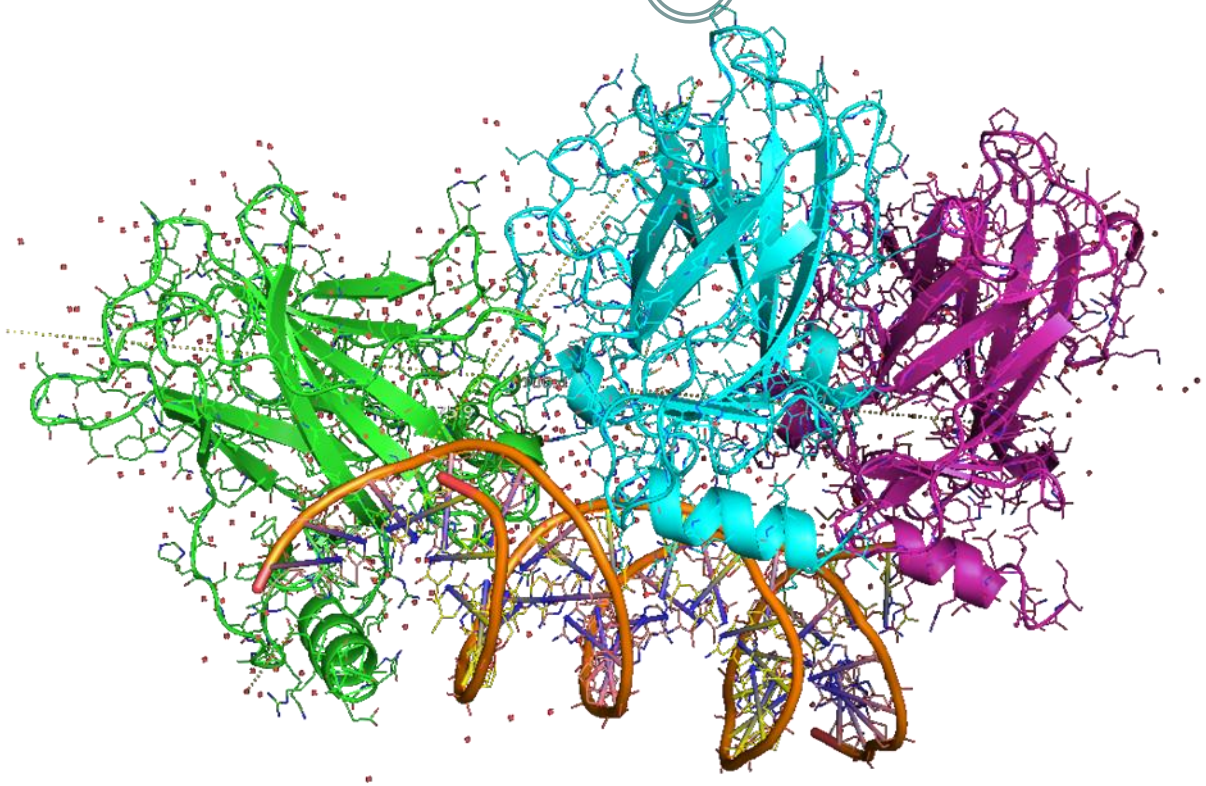


These processes occur at a higher rate in some cancer cells than they do in normal cells. Correspondingly, the rate of tumor suppressor gene loss is frequently higher in cancer cells than in pre-cancerous precursors.

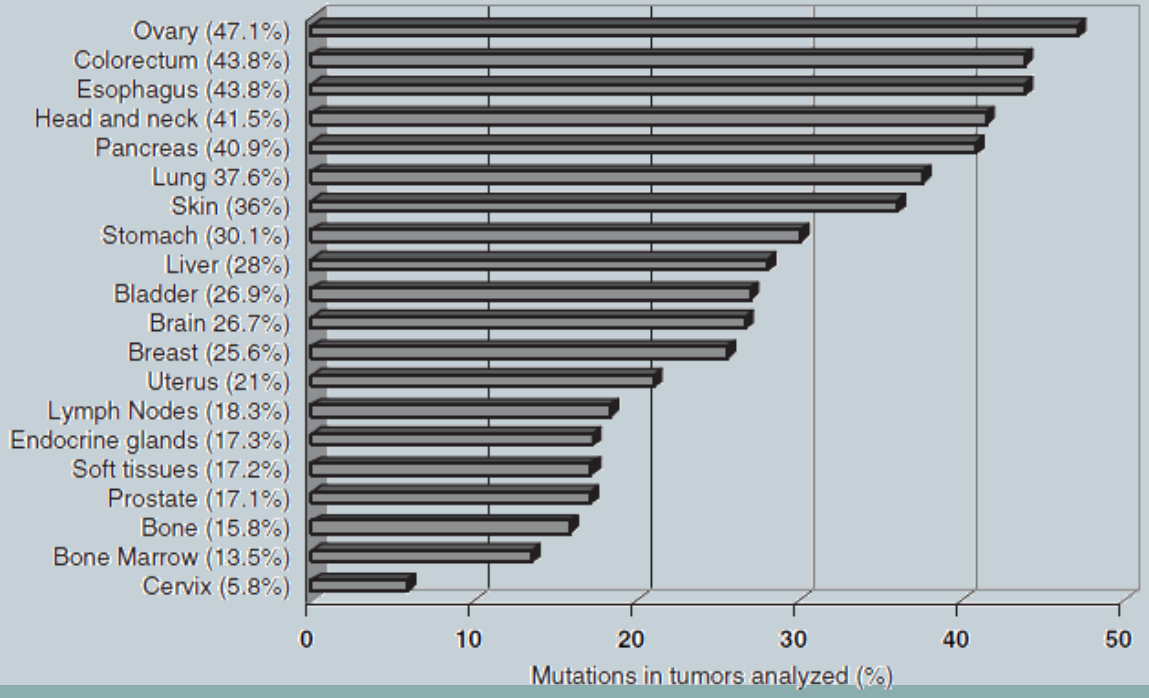
Recessive Genes, Dominant Traits

- Individuals are strongly predisposed to retinoblastoma if they inherit a single defective *RB allele*.
- *In general, while tumor suppressor genes are recessive, the* inheritance of a mutation in a tumor suppressor gene confers cancer susceptibility, which is a dominant trait.

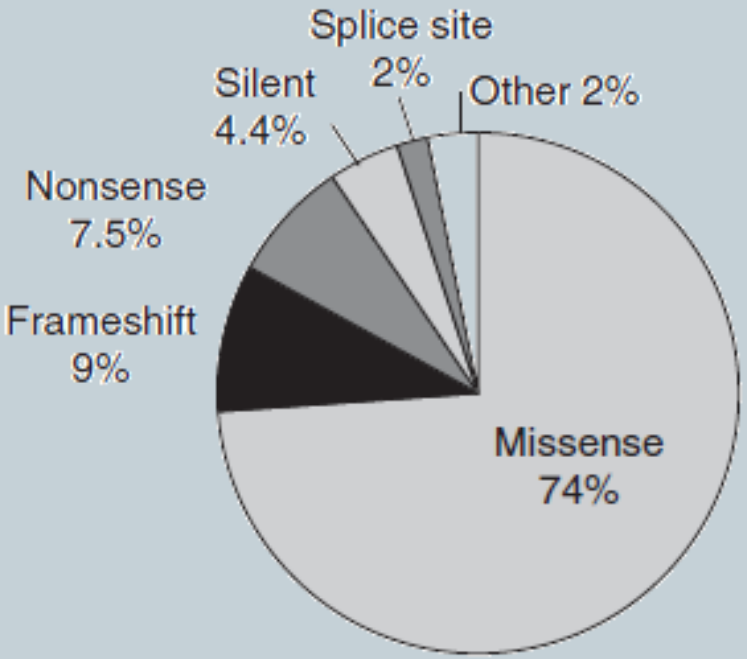
P53 Inactivation: A Frequent Event in Tumorigenesis



- Subsequent analysis of *P53 genes in large numbers of tumors revealed that* loss of *P53 is a frequent event in human cancers. A significant proportion of* tumors arising in many different tissues carry somatic mutations that inactivate *P53. Among tumors in which P53 mutations are prevalent are some* of the most common forms of cancer. Overall, *P53 is mutated in about half of all* human cancers.



- In contrast to *RB, which tends to be inactivated by large deletions, P53 is typically* inactivated by small alterations. A smaller proportion of mutations inactivate *P53 by truncating the open reading frame, either by a nonsense* point mutation or by a small insertion or deletion that causes a frameshift.
- In some cancer types, specific mutations in *P53 can be correlated with environmental mutagens*. Ultraviolet radiation, food-borne toxins, and cigarette smoke have all been found to leave highly characteristic mutations in *P53*.



- In a significant number of cancers, *P53 is inactivated not by mutation, but by the* activation of an antagonistic oncogene.
- This form of inhibition occurs at the posttranslational level and is mediated by protein–protein interactions.

Functional Inactivation of p53: Tumor Suppressor Genes and Oncogenes Interact

Two highly illustrative oncoproteins that inhibit p53 and contribute to tumorigenesis:

- *Mdm2*
- *Human papillomavirus oncoprotein E6 (HPV E6)*

Mdm2

- *MDM2 is a proto-oncogene that was originally found in double* minutes in tumorigenic mouse cells. The human homolog (sometimes called Hdm2) is an enzyme that covalently modifies proteins by the addition of ubiquitin.
- The ubiquitination of proteins by ubiquitin ligases like Mdm2 serves to target those proteins for degradation by the proteosome. Thus, the interaction of p53 with Mdm2 leads to p53 degradation, keeping p53 protein levels within a narrow range of intracellular concentration. In several types of cancers, principally soft-tissue sarcomas, the *MDM2 gene is amplified. The increased levels of Mdm2* are oncogenic, causing decreased levels of p53 and resulting in a loss of p53 function.
- *MDM2 is amplified in roughly one third of sarcomas. The MDM2 locus is* often amplified 50-fold or greater in these cancers.

HPV E6

- A second oncogene that affects p53 is not a cellular gene, but rather is a viral introduced upon infection by the human papillomaviruses (HPV). While the vast majority of cancers arise solely as a result of germline and/or somatically acquired mutations, an atypical exception is cancer of the uterine cervix. In the majority of cervical cancers, p53 is functionally inactivated by the inhibitory binding of an HPV-encoded protein known as E6.

Germline Inheritance of Mutant *P53*

Li–Fraumeni Syndrome

- LFS was first recognized as a clinical entity by Frederick Li and Joseph Fraumeni, in 1969.
- *P53 mutant alleles are found in the germline of individuals with an inherited* susceptibility to cancer known as Li Fraumeni syndrome (LFS).
- LFS is an autosomal dominant disorder mainly characterized by the early onset of bone or soft tissue sarcomas.

- The patterns of cancer that occur in LFS patients are partially dependent on the precise *P53 mutation inherited*.
- *Mutations within the exons that encode the DNA binding* domain of p53 are associated with a higher prevalence of brain tumors and an earlier onset of breast cancers, whereas mutations outside the DNA-binding domain are associated with a higher incidence of adrenal cancers.

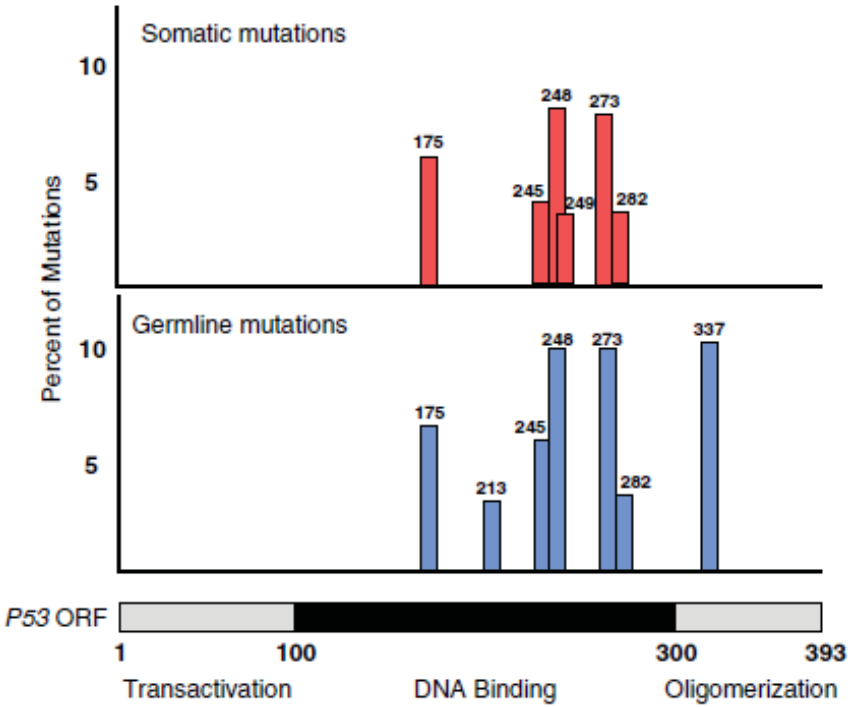


Fig. 3.10 Somatic and inherited mutations of *P53*. The distribution of *P53* mutations in Li Fraumeni kindreds and in sporadic tumors is similar. Most mutations affect the central region that encodes a sequence-specific binding domain critical for protein function. A highly acidic domain that interacts with other transcription factors is rarely targeted by mutation. A c-terminal domain is important for the organization of p53 molecules into active, oligomeric complexes. A relatively common germline mutation in this coding region is rarely found in sporadic tumors. Note that only the most common mutations (>3%) are shown. More rare mutations are generally clustered in the DNA-binding domain

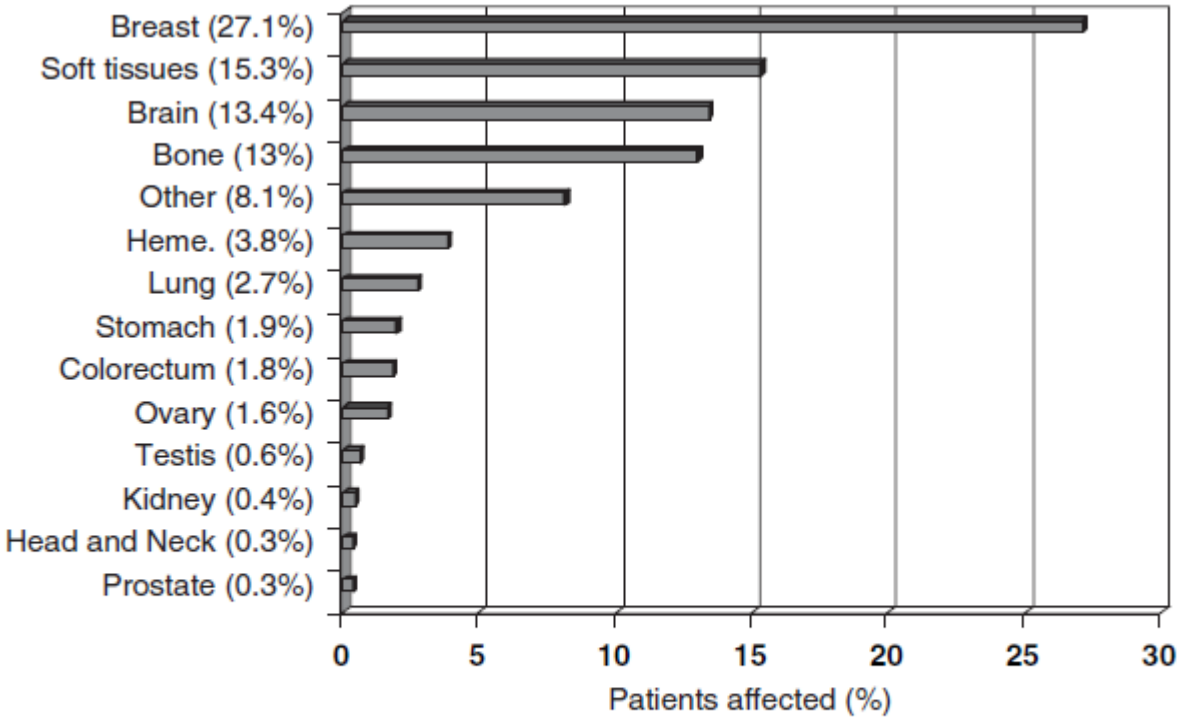


Fig. 3.11 Tumors associated with germline *P53* mutations. Individuals with Li Fraumeni syndrome are predisposed to diverse cancers. (Data from the IARC TP53 mutation database, R10 release, July 2005.)

Table 3.1 Tumor suppressor gene mutations in inherited cancer syndromes and in sporadic cancers. The predominant forms of inherited cancers are indicated in bold type

Gene	Cancer syndrome	Penetrance*	Inherited cancers	Sporadic cancers
RB	Familial retinoblastoma	>95%	Retinoblastoma Osteosarcoma Neuroblastoma Melanoma	Retinoblastoma Endometrial Bladder Osteosarcoma, Lung
APC	Familial adenomatous polyposis, Gardner's syndrome	>95%	Colorectal Osteosarcoma Small intestinal Gastric	Colorectal Gastric Small intestinal Adrenal gland Pancreatic
P53 (TP53)	Li Fraumeni syndrome	>95% females; ~75% males	Breast Sarcoma Brain tumors Osteosarcoma	Ovarian Colorectal Ca Esophageal Head and neck Pancreatic Lung Skin Breast Endometrial Lymphoma
PTEN (MMAC1, TEP1)	Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome	>95%	Breast Thyroid Endometrial Brain	Endometrial Brain Prostate Lung Breast Bladder Ovarian Lymphoma

BRCA1	Familial breast and Ovarian Ca	~80% breast; ~40% ovarian	Breast Ovarian	Ovarian Breast (rare)
BRCA2	Familial breast Ca	~80% breast; ~20% ovarian	Breast (inc. males) Ovarian Pancreatic	Breast (rare) Colorectal (rare)
NF1	Neurofibromatosis Type 1	>95%	Brain Neural tumors	Melanoma Neuroblastoma
NF2	Neurofibromatosis Type 2	>95%	Neural tumors	Brain tumors
VHL	von Hippel-Lindau syndrome	>60%	Kidney Hemangioblastoma	Kidney Hemangioblastoma
MEN1	Multiple endocrine neoplasia	~90%	Pancreatic islet Cell	Pituitary Adenomas Parathyroid
SMAD4 (DPC4)	Familial juvenile polyposis syndrome	~20%	Colorectal Gastric Small intestinal	Pancreatic Colorectal
CDKN2A (P16, INK4, MTS1)	Familial melanoma	~70%	Melanoma Pancreatic Breast	Melanoma Pancreatic Esophageal Lung Head and neck Leukemia Bladder
MSH2, MLH1, MSH6, PMS2	Hereditary nonpolyposis colorectal cancer, Turcot syndrome	~80% colorectal; ~70% endometrial	Colorectal Endometrial Ovarian Small intestinal Bladder Brain Biliary tract	Colorectal Gastric Endometrial Bladder

[a]Lifetime risk for developing the predominant form of cancer.

Relative risk

- *All human beings are at risk of cancer. In kindreds with germline* tumor suppressor gene mutations, that risk is elevated. For a given cancer, the relative risk (also known as the risk ratio) compares the probability of cancer in two groups and is defined as:

- Relative risk = Absolute risk of cancer in carriers (%) / Absolute risk of cancer in the general population (%)

Odds ratio

- Another comparison of risk between two cohorts is the odds ratio. Most often applied to case-control studies in which the outcome (i.e. cancer) is a rare occurrence, the odds ratio compares the relative odds of cancer between two groups. Applied to the analysis of carriers of a specific allele:
- Odds ratio =

Odds against developing cancer in the general population / Odds for developing cancer in carriers

- As an example, consider a hypothetical cancer-causing allele that has a penetrance of 10%. If the incidence of the same cancer in the general population is 1%, the relative risk is 0.10/0.01 = 10. In contrast, the odds ratio would be (99 to 1)/(1 in 10) = 99/0.1 = 990. In general, the relative risk yields a more intuitive result than an odds ratio, but can lead to misleading results if applied to studies in which only the outcome is measured.

Breast Cancer Susceptibility: *BRCA1 and BRCA2*

Table 3.2 Lifetime risks for developing cancer associated with *BRCA1* and *BRCA2* mutations. The penetrance of *BRCA1* and *BRCA2* mutations has been found to be highly variable. Figures shown are representative but highly approximate

		Mutant *BRCA1* carrier		Mutant *BRCA2* carrier	
Cancer	General population	Risk	Relative risk*	Risk	Relative risk*
Breast	12%	80%	6.7	80%	6.7
Ovarian	1.8%	40%	22	20%	11
Male breast	0.1%	3%	30	6%	60

*Defined as the fold-increase in the overall risk attributable to the mutated gene.

Most Tumor Suppressor Genes are Tissue-Specific

Inherited Tumor Suppressor Gene Mutations

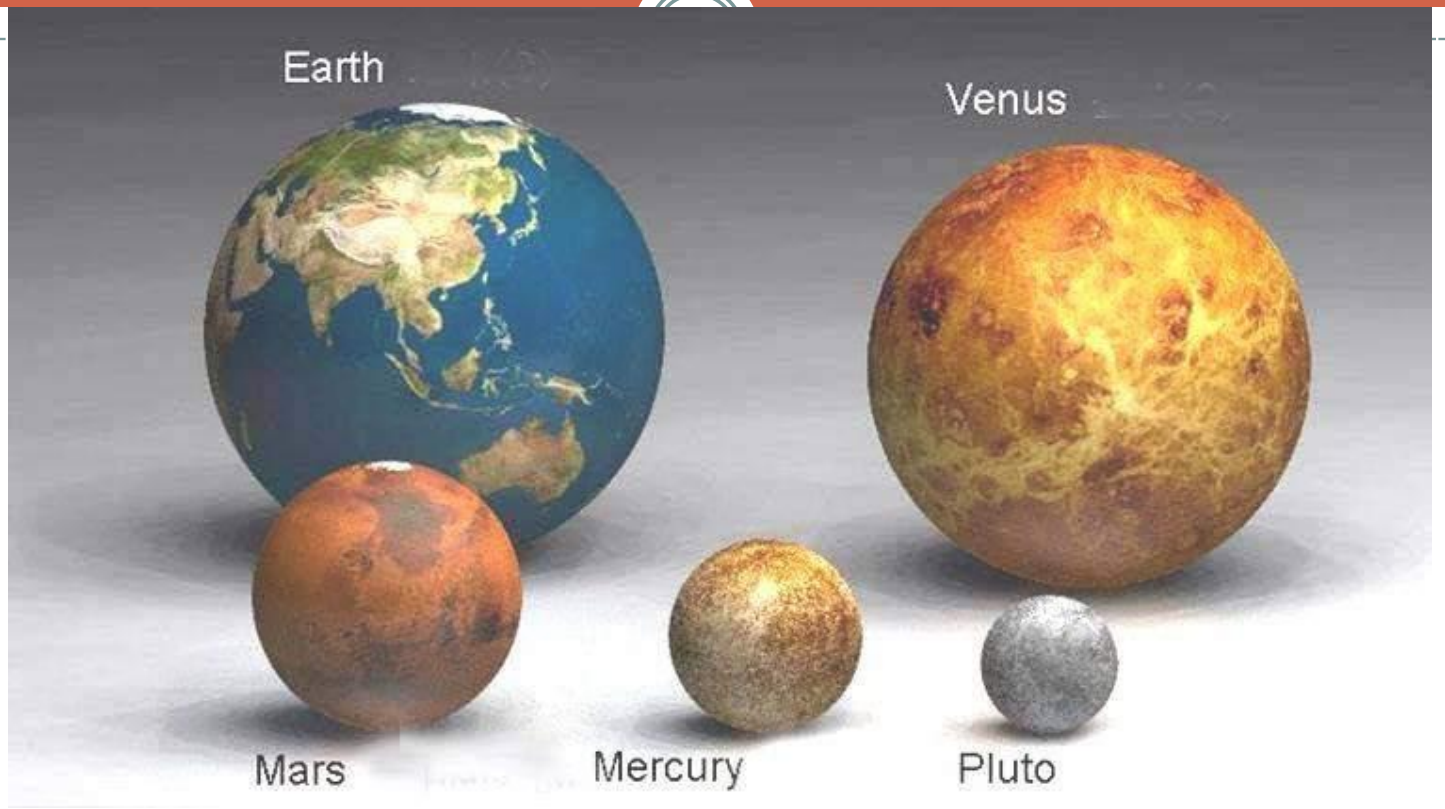
- **Caretakers:** encode products that stabilize the genome. Fundamentally, mutations in caretaker genes lead to genimic instability. Tumor cells arise from two distinct classes of genomic instability: mutational instability arising from changes in the nucleotide sequence of DNA and chromosomal instability arising from improper rearrangement of chromosomes.

- **Gatekeepers:** encode gene products that act to prevent growth of potential cancer cells and prevent accumulation of mutations that directly lead to increased cellular proliferation

- **Landscapers:** encode products that, when mutated, contribute to the neoplastic growth of cells by fostering a stromal environment conducive to unregulated cell proliferation.

- Inactivated *APC alleles are highly penetrant while inactivated SMAD4 alleles* are less so.
- Classical growth-controlling tumor suppressor genes such as *APC have been* categorized as '*gatekeepers'. Gatekeepers directly suppress cell outgrowth. Cells* that lose gatekeeper activity form neoplasia, each of which has the potential to become a cancer. When wild type gatekeeper genes are experimentally reintroduced into established cancer cells, they typically lead to suppression of growth.
- The inherited mutations of *SMAD4 affect epithelial cell populations in a less* direct manner. Germline *SMAD4 mutations appear to primarily alter the growth of* stromal cells that are not cancer precursors. *SMAD4 inactivation thus alters the* tissue structure of the colorectum. This abnormal microenviroment provides a fertile landscape for the outgrowth of epithelial neoplasia. Mutations in *SMAD4* typify what has been termed a '*landscaper' defect*.

- *RB clearly functions as a gatekeeper in the cells of the developing retina.*
- In contrast, the timing of *P53 inactivation in later stages of colorectal cancer suggests* that *P53 does not function as a gatekeeper in the colorectal epithelium.*
- However, a significant body of evidence suggests that *P53 is a gatekeeper in other* cancer types, most notably breast cancers.
- Examples of caretaker genes are *BRCA1 and BRCA2, breast cancer susceptibility genes that are required* for DNA repair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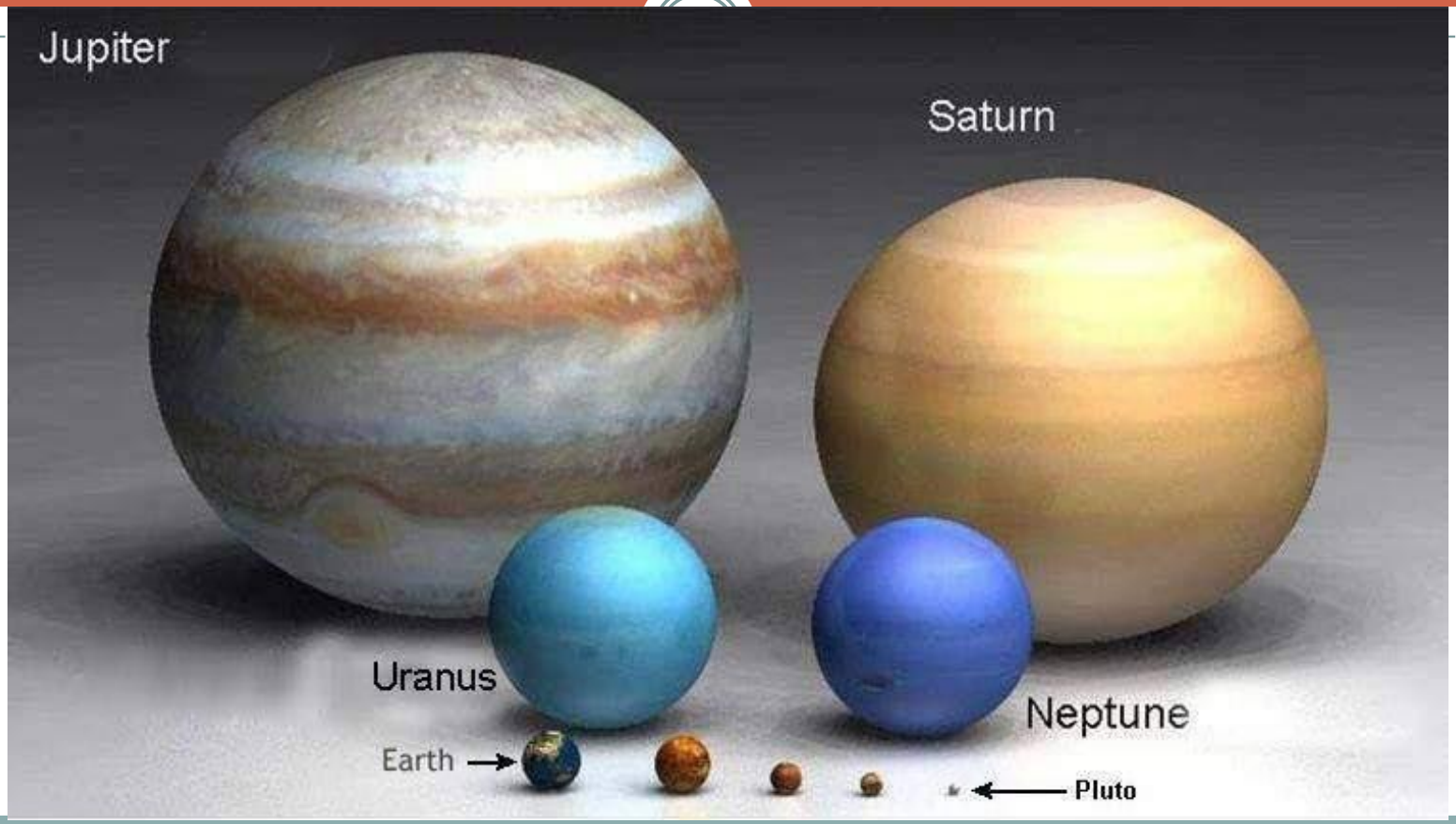
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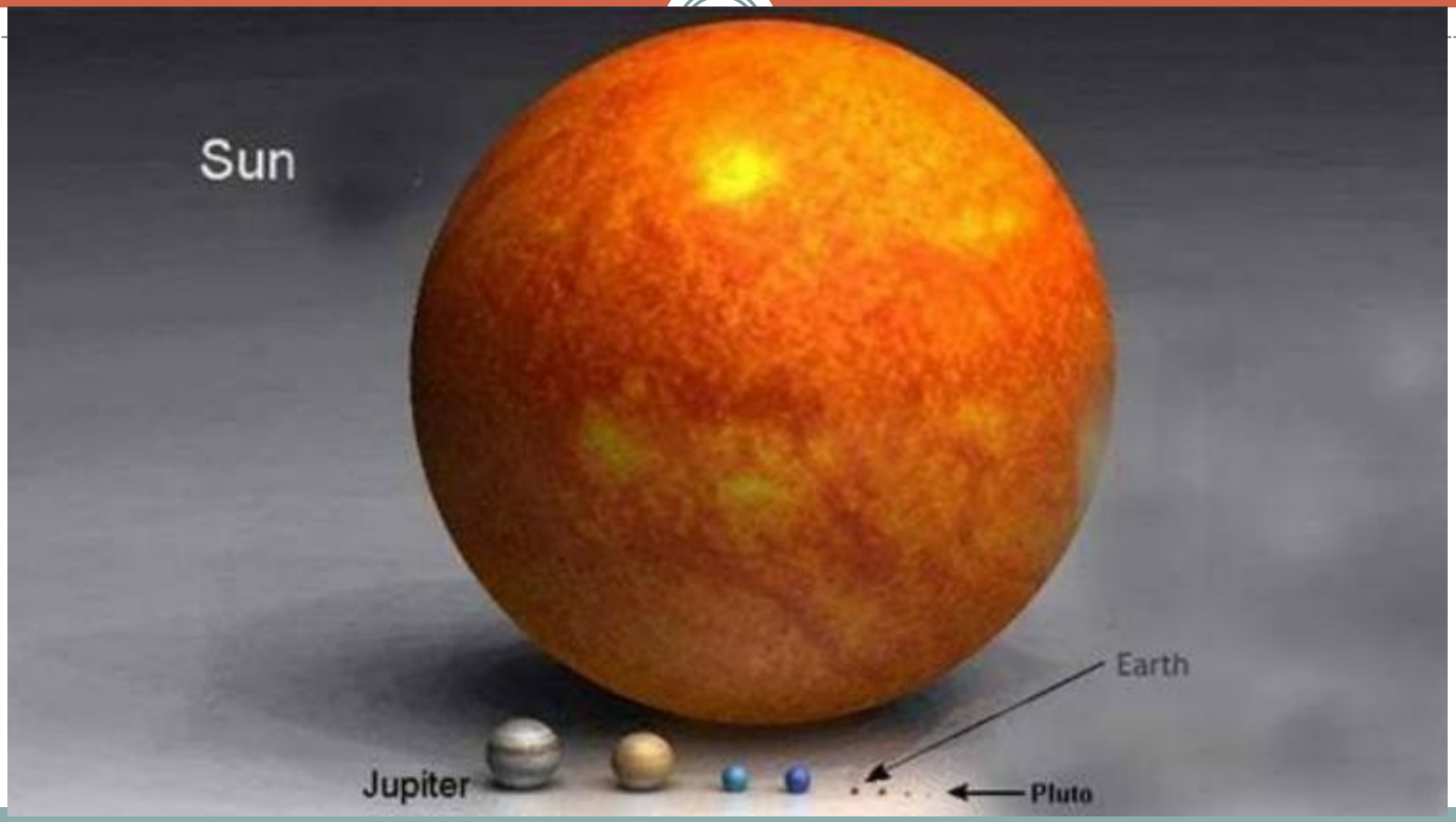
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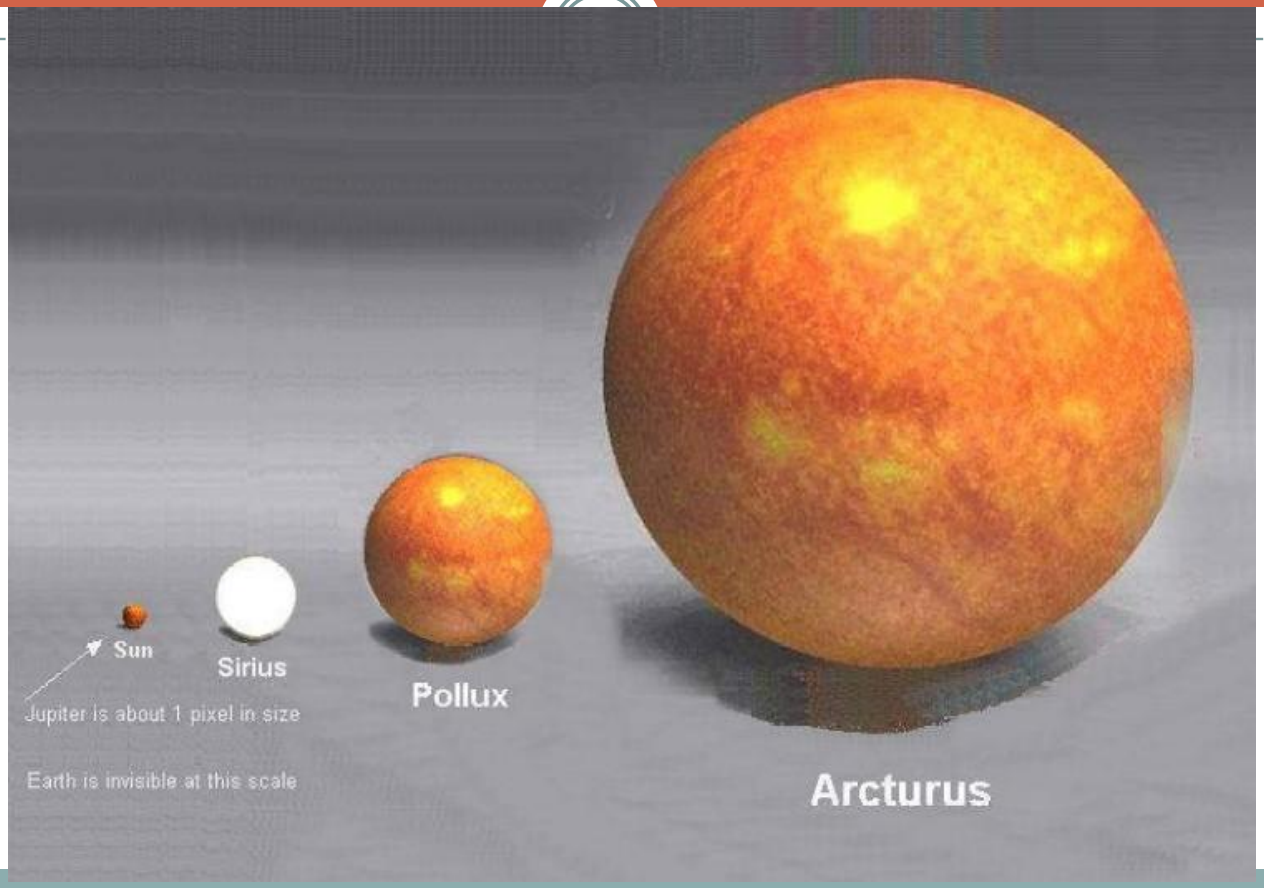
Sun
Sirius
Pollux
Arcturus
Jupiter is about 1 pixel in size
Earth is invisible at this scale


Antares
Betelgeuse
Arcturus
Rigel
Aldebaran