TOPIC: MECHANISMS OF CANCER FORMATION

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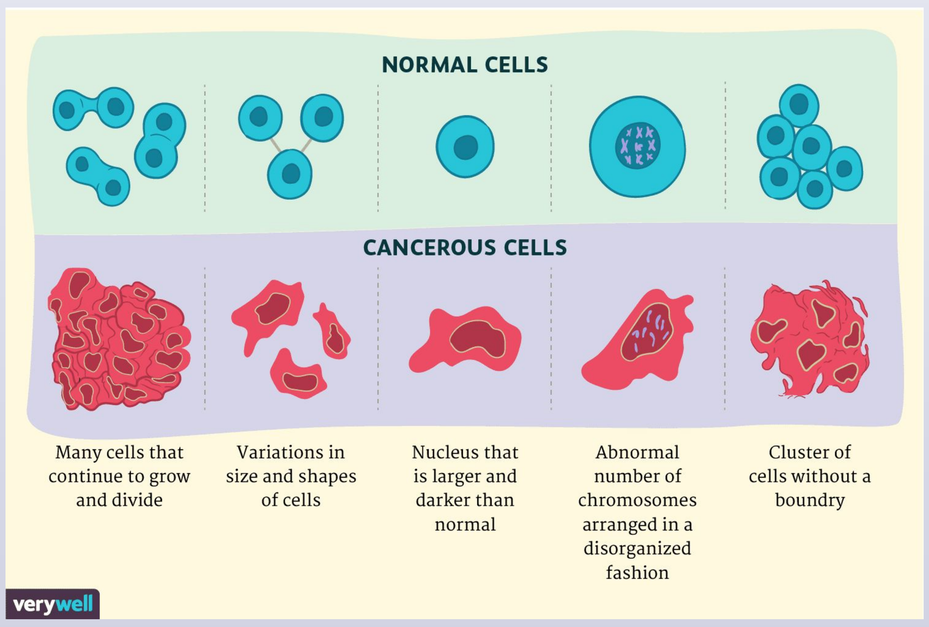
How does cancer emerge in human body?

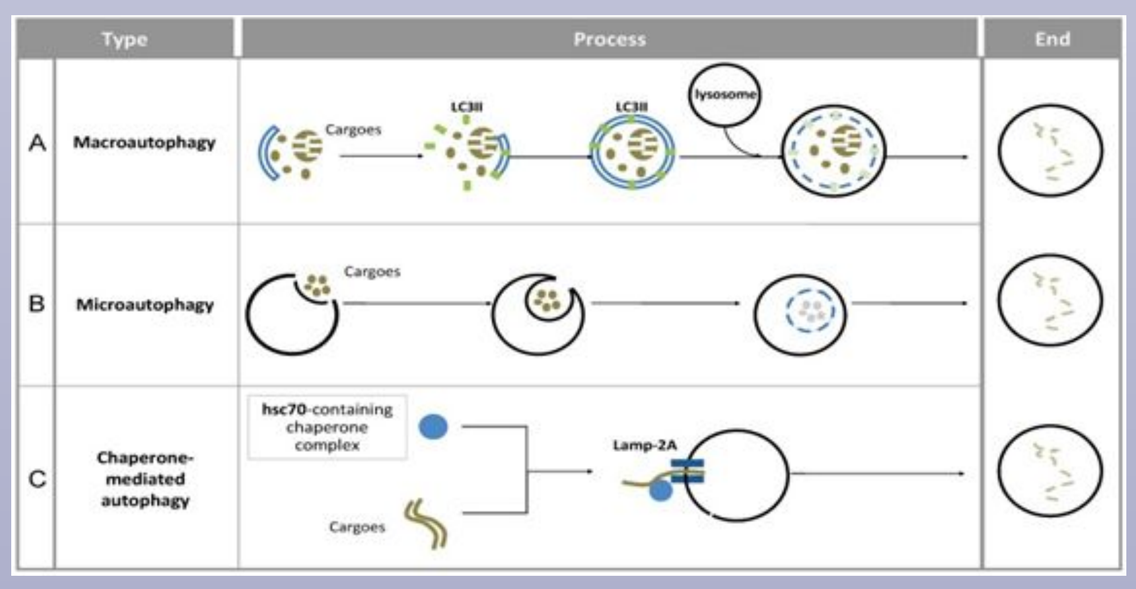
- Body cells start to divide uncontrollably and can emerge in any part of the body.
- Cancer is a genetic disease. Changings on the genes that are responsible for reproduction and development cause cancer.
- Cancer cells are having more genetic alteration than normal body cells.
- Cancer cells undergo rapid mitosis without programmed cell death and are stuck in the G0 phase or may experience shorter time duration within the G1 phase.



What is autophagy?

- Autophagy is the process of self-digestion to maintain the energy balance in cell. All structures of the cell are broken down and recycled in a controlled manner. For the transaction, lysosome organelle crashes most of the cell components.

Types of Autophagy



1. Macroautophagy
2. Microautophagy
3. Chaperone - mediated Autophagy

Autophagic Role

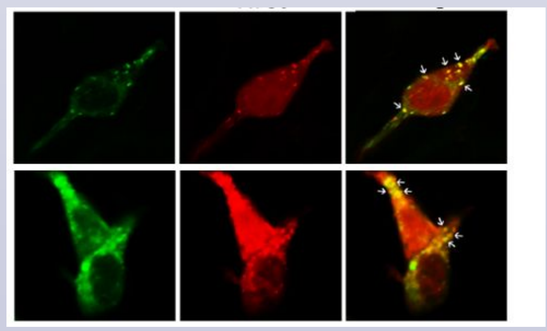
- During the initial stages of tumorigenisis, autophagy may exhibit tumor supressor functions by (i) limiting chromosomal instability (ii) restricting oxidative stress (iii) preventing intratumoral necrosis and local inflammation in response to metabolic stress. In the later stages of tumorigenisis, the Autophagic activity shifts towards the tumor promoting functions under the metabolic stress, metastasis and chemotherapy.

Studying Autophagic Activity

Procedures:

1. Fluorescent Microscopy

Under the fluorescence, the number of Autophagic vacuoles was counted in HEK Cells and MCF VII Cells in each field of view to determine how the Cytokines had affected the rate of Autophagy within the cells. GFP LC3 was used a gene marker, once the expression of this gene occurs; it helped visualization of Autophagosome formation under the fluorescence.



2. Luciferase Assay

The reporter protein's activity or fluorescence within a transfected cell population is approximately proportional to the steady-state mRNA level. A commonly used reporter gene is the luciferase gene from the firefly Photinus pyralis.Through PEI transfection, GFP LC3 gene is fused with MCF-7 cells, to successfully form MCF-7/Luc cells. This allows us to measure Cytokine activity through measuring luciferase activity under fluorescent light.

3. Western Blotting

The sample undergoes protein denaturation, followed by gel electrophoresis. A primary antibody is created that recognises and binds to a specific target protein. The electrophoresis membrane is washed in a solution containing the primary antibody, before excess antibody is washed off. A secondary antibody is added which recognises and binds to the primary antibody. The secondary antibody is visualised through various methods such as staining, immunofluorescence, and radioactivity, allowing indirect detection of the specific target protein.