RESEARCH REPORT

KIRAMS


KIRAMS
KOREA INSTITUTE OF RADIOLOGICAL & MEDICAL SCIENCES
SINCE 1963

Development of an epigenetic changes method to improve the efficacy of tumor radiotherapy

Research subject

- Expansion of biological infrastructure through radiation reaction research
- Establishment and validation of precise diagnosis of radiation therapy
- Radiation-resistant biomarker mass discovery and verification
- Development of precise diagnostic technology for patient-specific radiation therapy

Research contents

- Securing bio-data for establishment of radiation response map
  - Obtaining bio-energy bio-data for identification of radiation-specific metabolic function
  - Confirmation of mitochondrial respiration after muscle cell (C2C12) irradiation through XF assay
- Analysis of transcriptom/proteome/metabolome of radiation resistant cell lines
  - Observation of relationship between abnormal DNA methylation by radiation and radiation resistance and identification of molecular phenomena and markers contributing to the radiation in order to improve the effectiveness of radiation therapy

Research contents

- Identification of clinical bioindicators for radiation therapy
  - Diagnosis of energy metabolism change in human body through analysis of energy metabolism-related molecules and metabolites in radiation treatment process and prediction of sensitivity and adaptability of radiation to human body
- Discovery and verification of radiation-resistant biomarker
  - Development of technology for diagnosis of prognosis or improvement of radiation treatment

RESEARCH ASSISTANCE

GRADIENT PCR

→ determination of an optimal annealing temperature using the least number of steps

PCR

→ polymerase chain reaction – amplification of DNA sequences

CELL CULTURES

→ Cell plating, maintaining cells in a culture, cell passaging and media change

GEL ELECTROPHORESIS

→ separation and analysis of macromolecules and their fragments, based on their size and charge

MTT ASSAY

→ colorimetric assay for assessing cell metabolic activity

WESTERN BLOT

→ detection and analysis of proteins

XF ASSAY

- ➔ assay for measuring glycolytic function in cells

Measures:

- ➔ OCR – oxygen consumption rate
- ➔ ECAR – extracellular acidification rate

to check main resource of mitochondrial energy in cell
mito stress

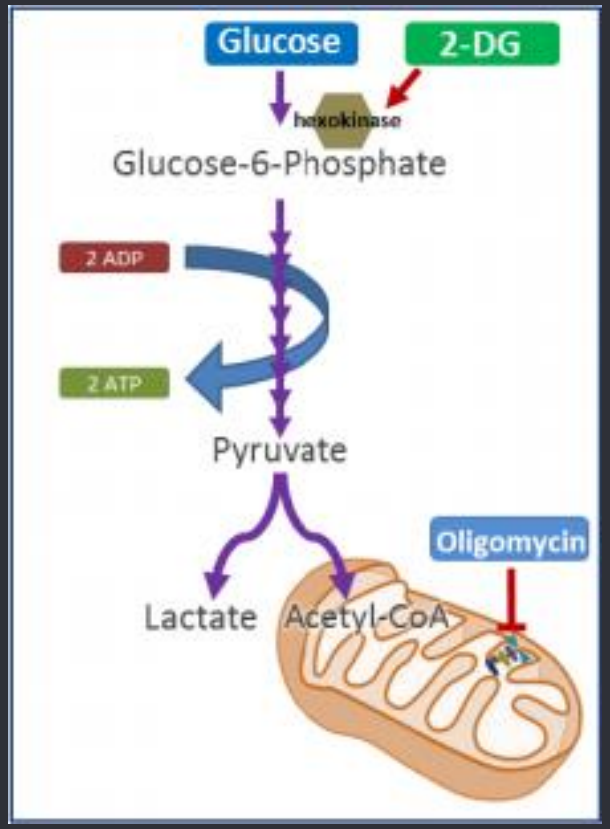
SEAHORSE XF GLYCOLYSIS STRESS TEST

- ➔ assay for measuring glycolytic function in cells

Measures:

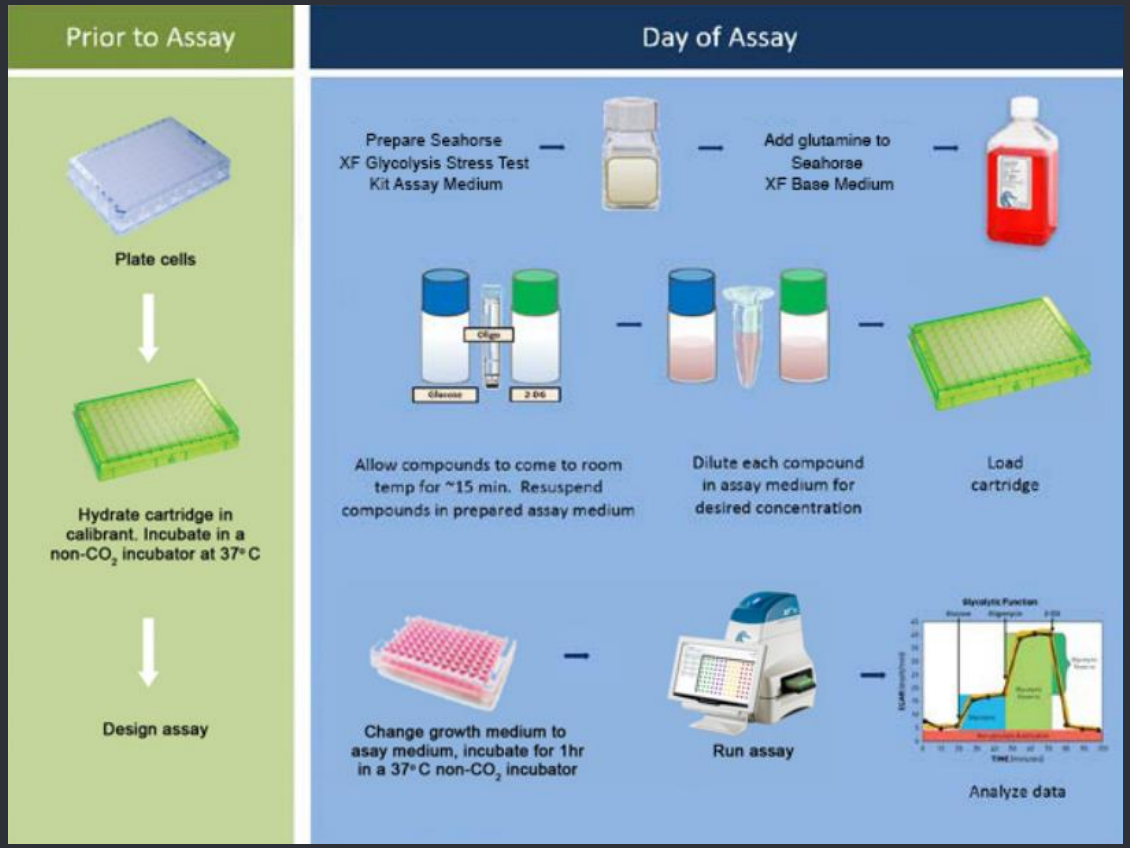
- ➔ OCR – oxygen consumption rate
- ➔ ECAR – extracellular acidification rate

Seahorse XF glycolysis stress test modulators of glycolysis



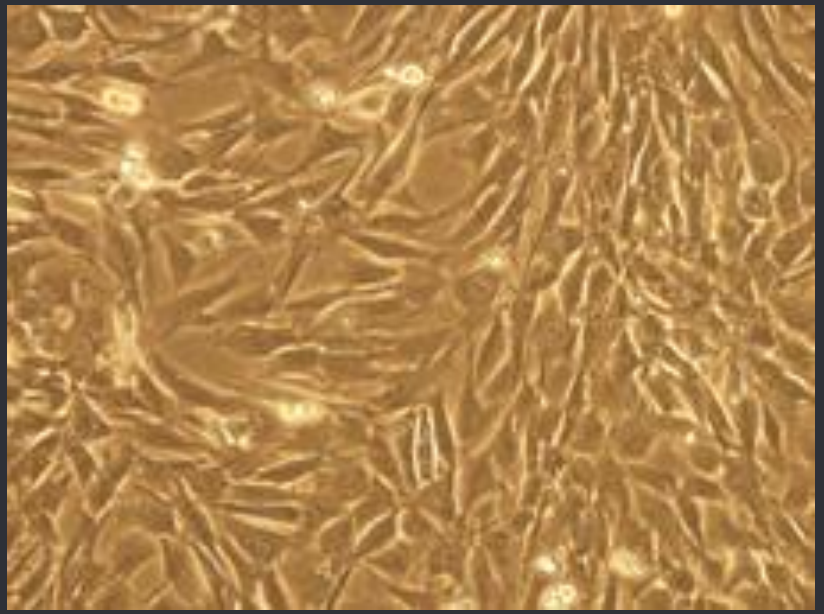
➔ Simplified version of glycolysis and the sites of action of the kit components

Seahorse XF glycolysis stress test assay workflow

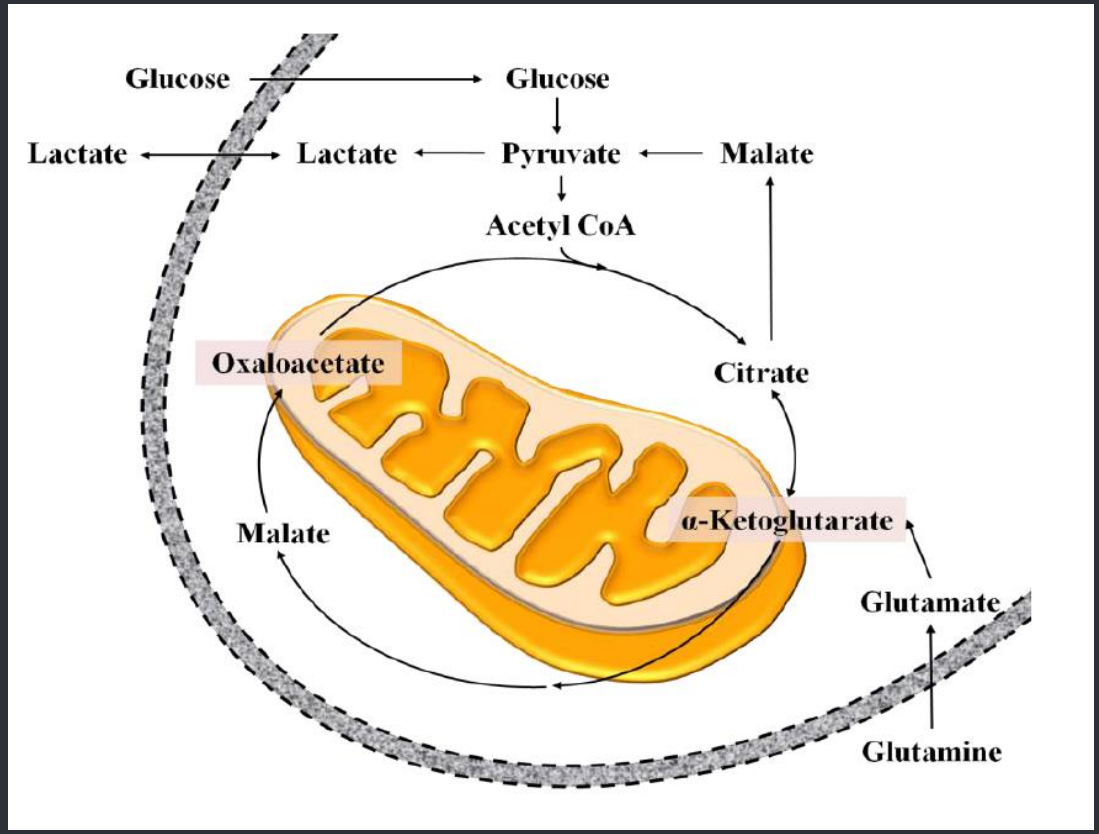


C2C12

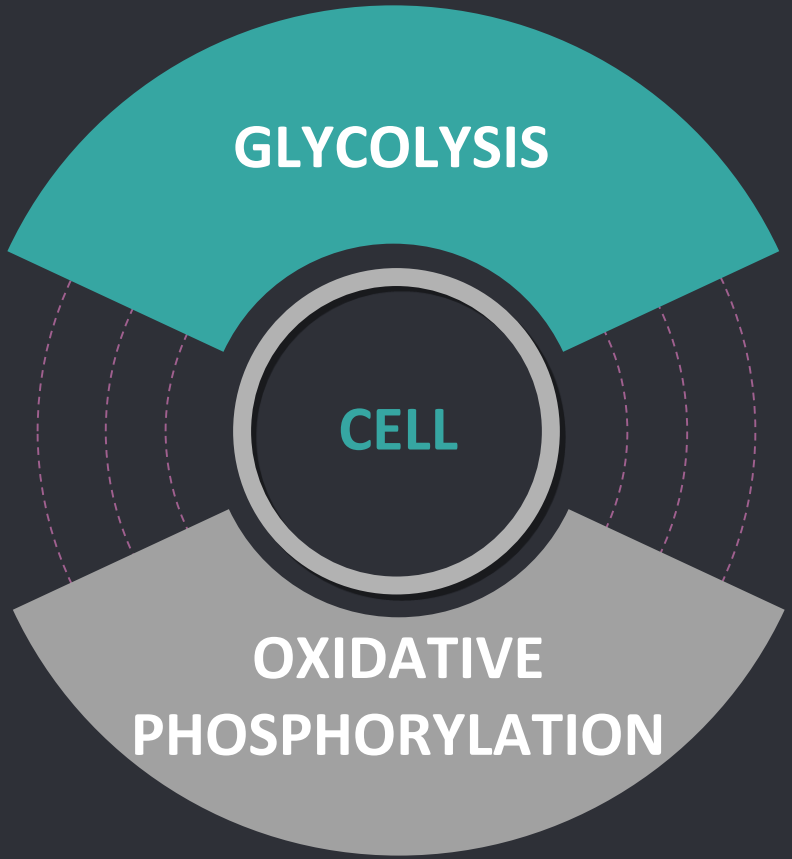
➔ immortalized mouse myoblast cell line



Mitochondrial respiration

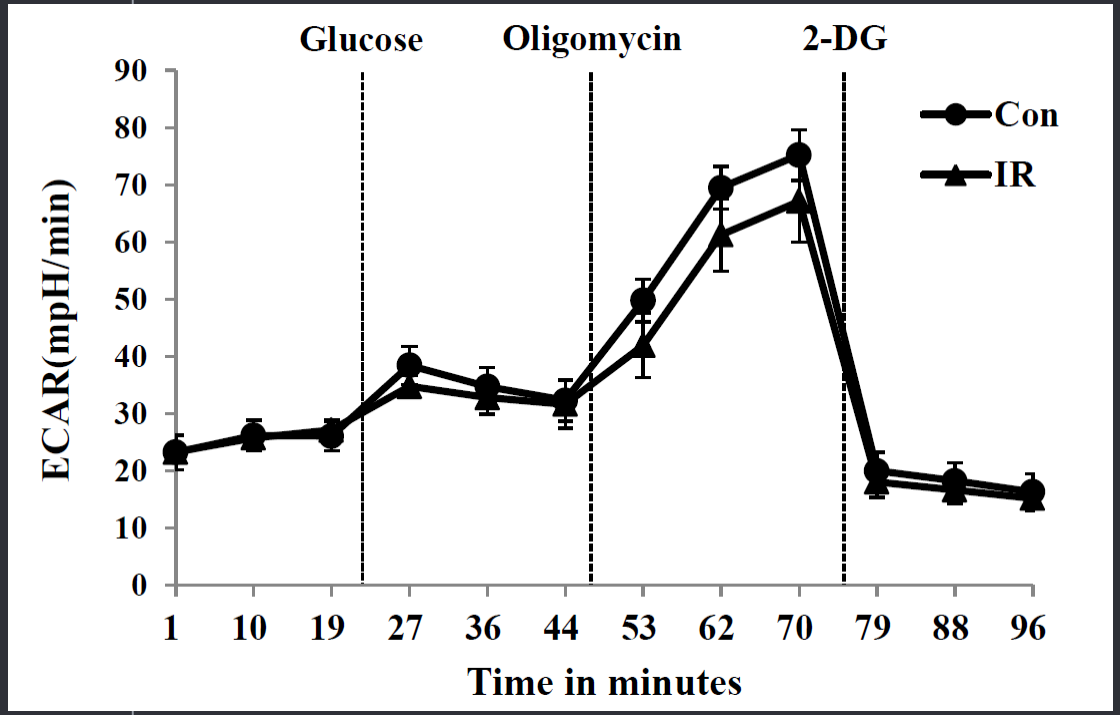


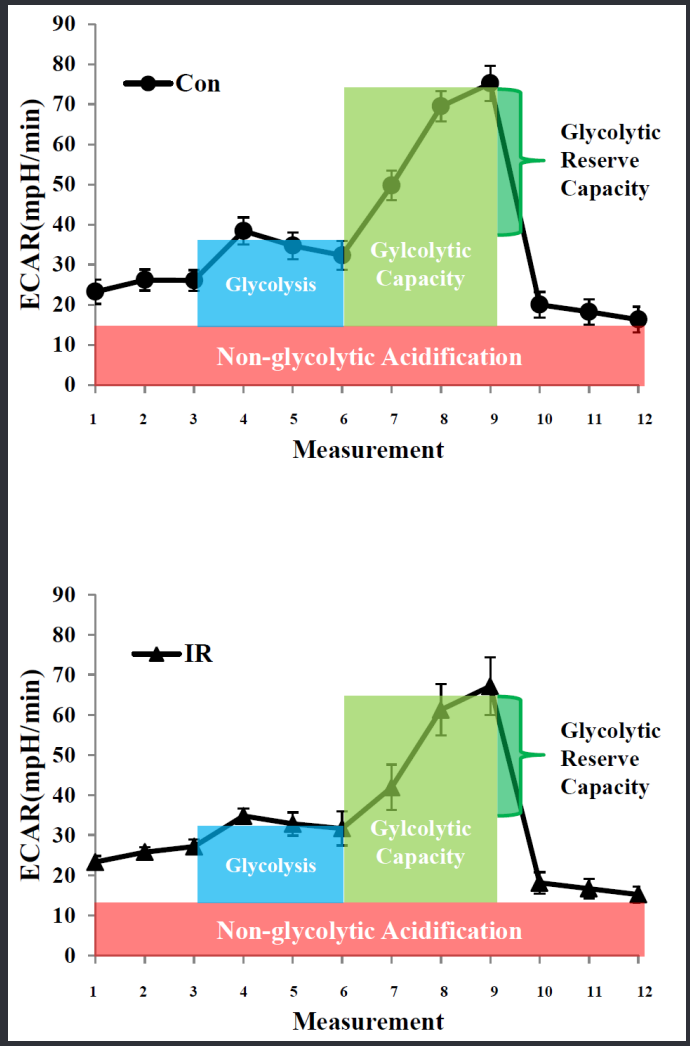
Major energy-producing pathways in the cell



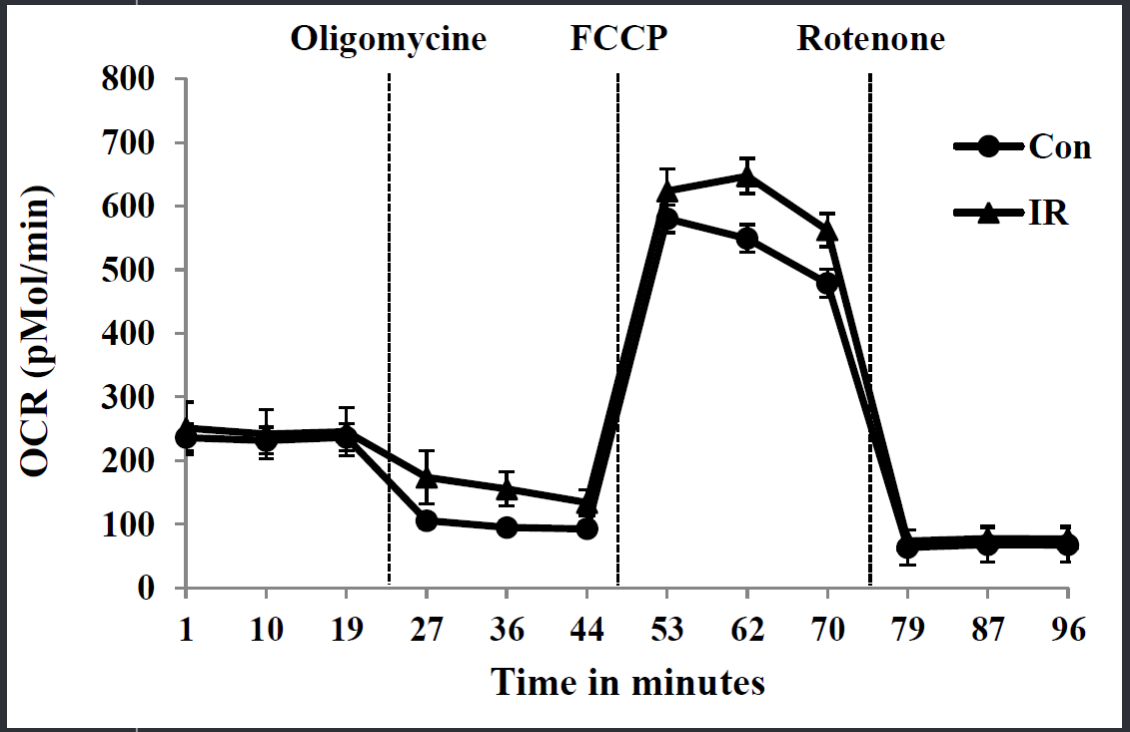
Main energy-producing resource in mitochondria?

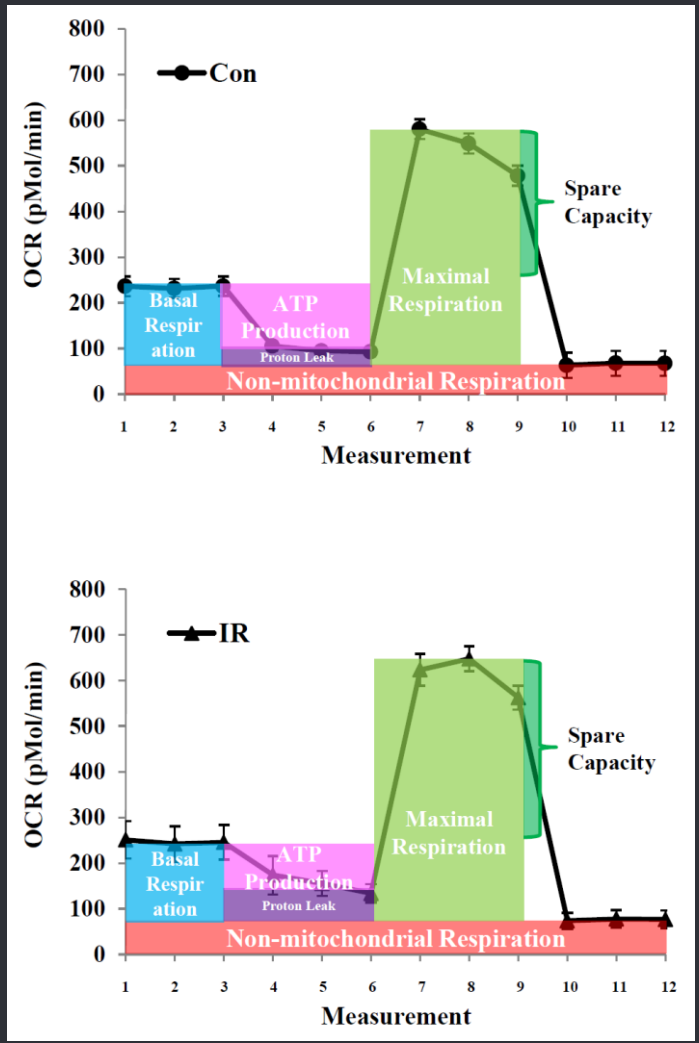
ECAR


Glucose
Oligomycin
2-DG
Con
IR
ECAR(mpH/min)
Time in minutes

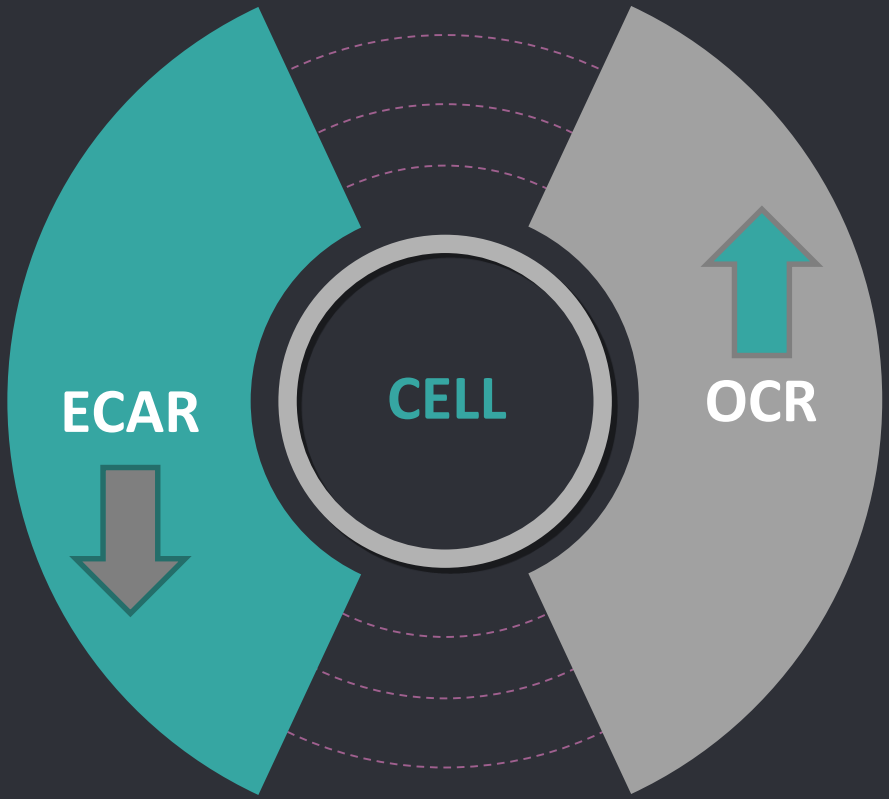

Con
IR
Glycolysis
Gylcolytic Capacity
Glycolytic Reserve Capacity
Non-glycolytic Acidification
ECAR(mpH/min)
Measurement

OCR


Oligomycine
FCCP
Rotenone
OCR (pMol/min)
800
700
600
500
400
300
200
100
0
1
10
19
27
36
44
53
62
70
79
87
96
Time in minutes
Con
IR


OCR (pMol/min)
Con
Basal Respir ation
ATP Production
Proton Leak
Maximal Respiration
Spare Capacity
Non-mitochondrial Respiration
Measurement
IR
Basal Respir ation
ATP Production
Proton Leak
Maximal Respiration
Spare Capacity
Non-mitochondrial Respiration
Measurement

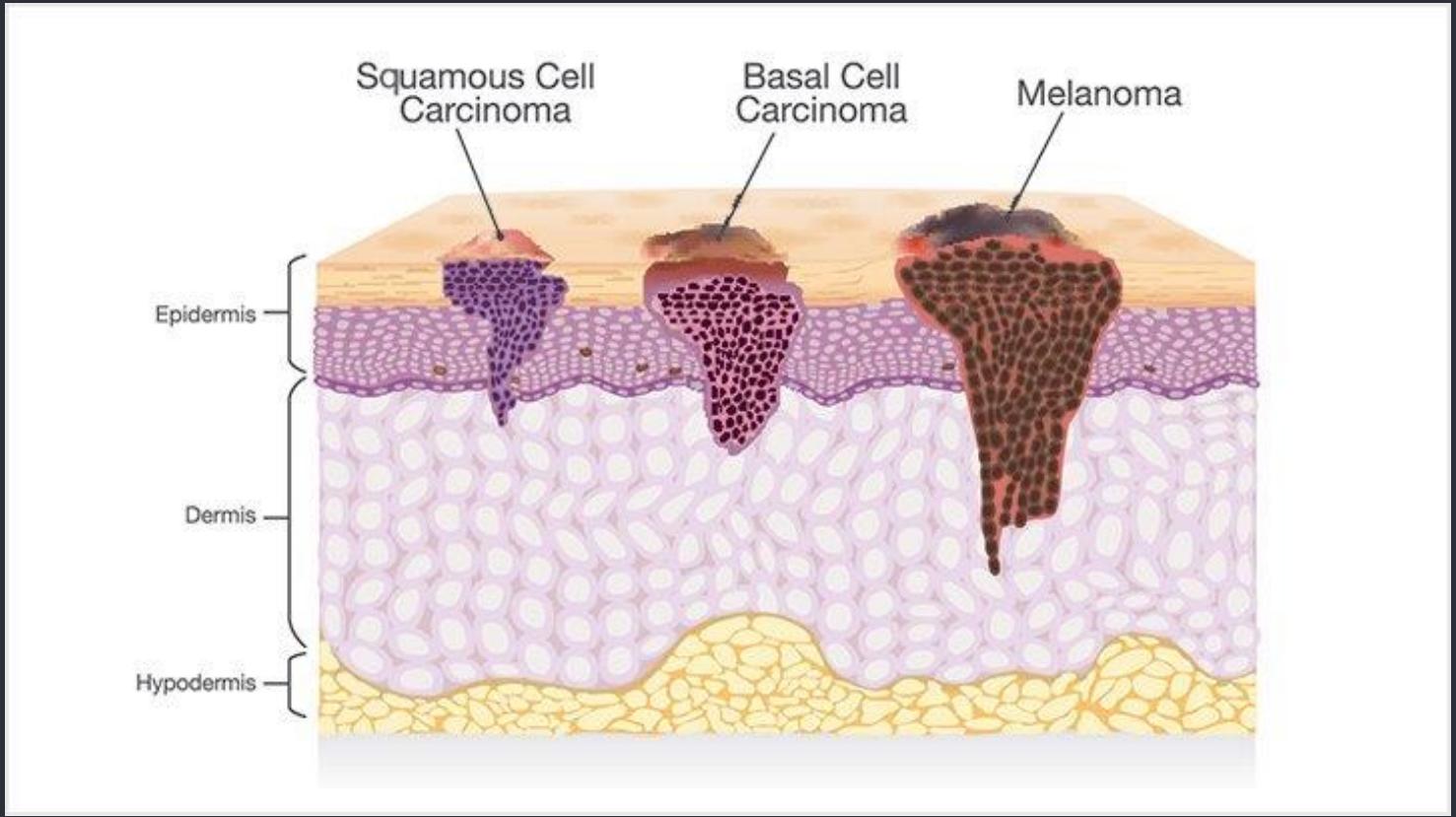
RESULTS



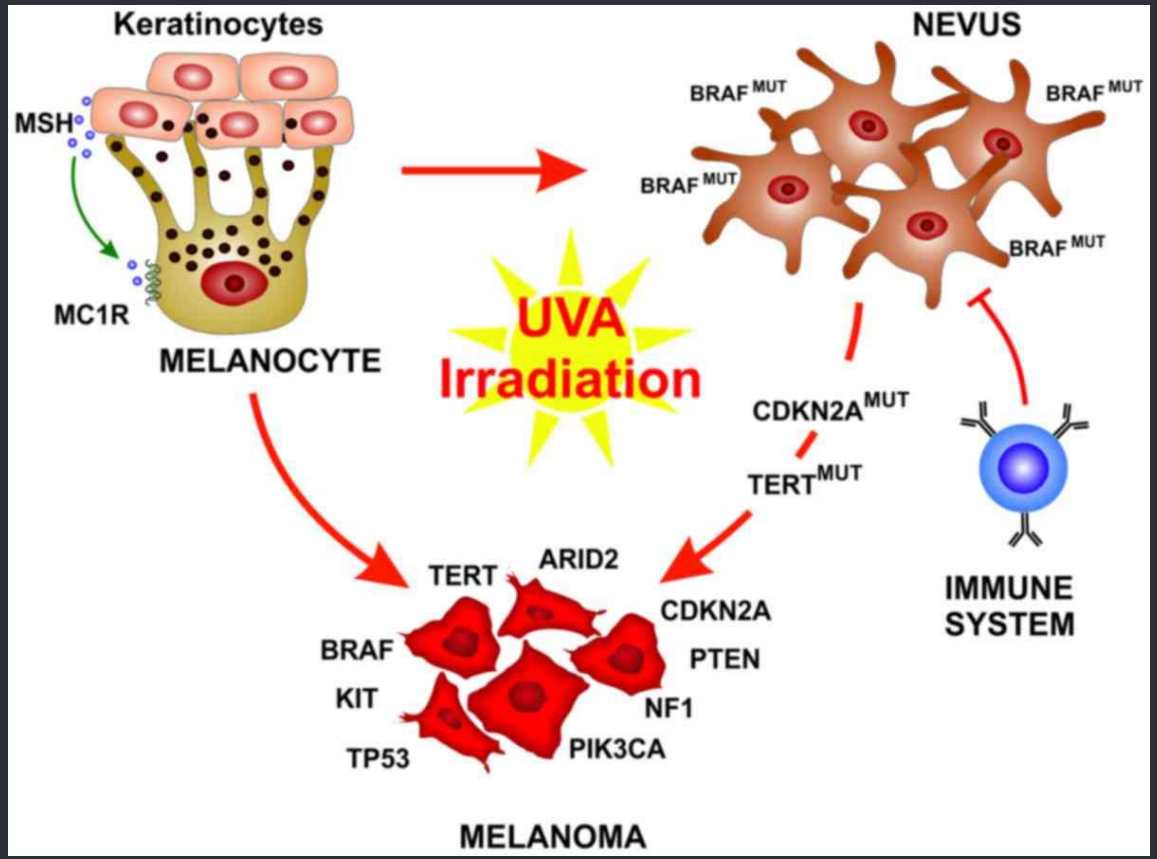
Further study

- Radiation effects on melanoma cells

Melanoma


Squamous Cell Carcinoma
Basal Cell Carcinoma
Melanoma
Epidermis
Dermis
Hypodermis

Melanocyte malignant transformation



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SUMMARY

- ➔ Radiotherapy development research
- ➔ Radiation influence on cancerous tissue (melanoma)
- ➔ Analysis of radiation-treated cells through XF assay