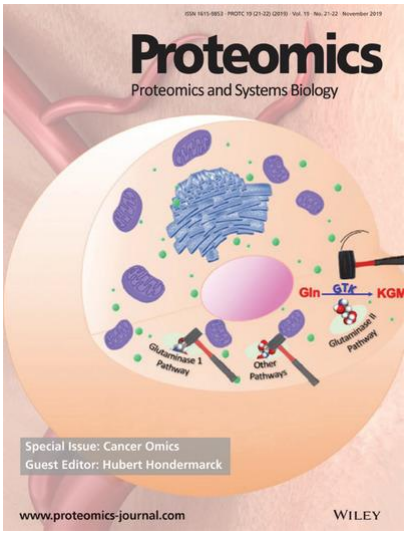
Proteomic analysis of stromal and epithelial cell communications in human endometrial cancer using a unique 3D co-culture model



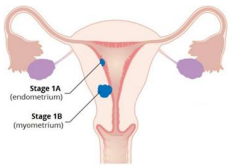
Endometrial cancer

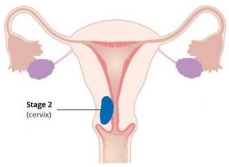
Endometrium: (the inner part of the uterus) undergoes many biological events across the menstrual cycle for maintaining the homeostasis and preparing for embryo implantation and established pregnancy. These events physiologically regulate by a sequentially timed interplay of female sex hormones and microenvironmental cues.

Endometrial cancer (EC): is the fifth leading cancer in women worldwide and second most common gynaecological cancer. Its incidence is higher in the Western world, accounting for 6-9% of all cancers in women

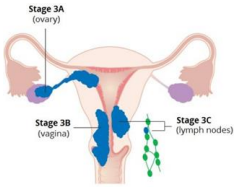
EC staging (International Federation of Obstetrics and Gynaecology (FIGO))

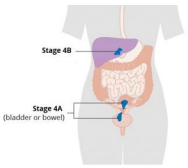
- Stage I – Carcinoma confined to the uterus





- Stage II – Carcinoma extend to cervix but is not beyond the uterus
- Stage III – Carcinoma extends beyond uterus to the vagina, ovaries and/or lymph nodes



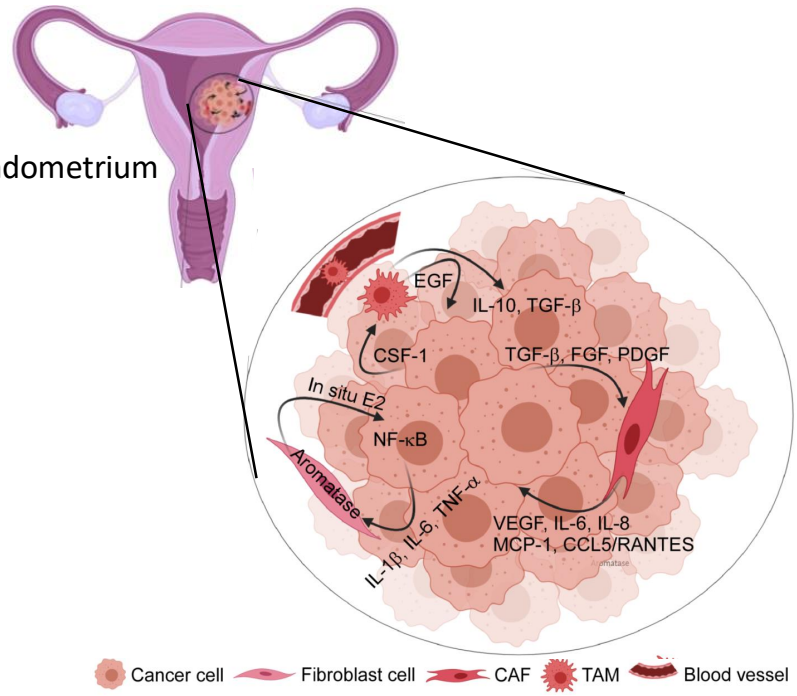


- Stage IV – Carcinoma involves urinary bladder or bowel, or has metastasized to distant sites

Pathogenesis of endometrial cancer

- ❑ Unopposed exposure to estrogen that induced mitotic division in endometrial cells and frequently end up to DNA replication errors and somatic mutations
- ❑ Role of stromal cells in boosting the tumorigenicity of endometrium

- ➢ Fibroblast
  - IL-1 β, IL-6, TNF-α
  - In situ estrogen biosynthesis
- ➢ Cancer associated fibroblast (CAF)
  - TGF-β, FGF, PDGF
  - VEGF, IL-6, IL-8, chemockines
- ➢ Tumour associated macrophages (TAM)
  - CSF-1
  - EGF, IL-10, TGF-β



Diagnosis endometrial cancer

Endometrial cancer diagnosis

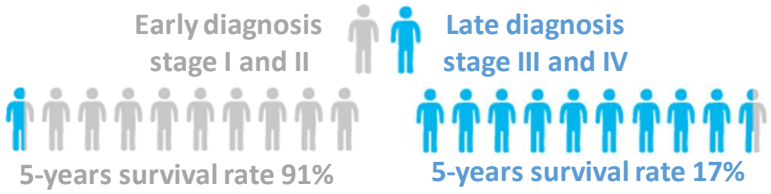
- Symptoms
  - Vaginal bleeding or discharge not related to menstruation
  - Vaginal bleeding after menopause
  - Difficult or painful urination
  - Pain during sexual intercourse
  - Pain in the pelvic area

- Modalities of Endometrial Cancer Screening
  - CA-125
  - CT&MRI for staging the Endometrial Cancer
  - Hysteroscopy, endometrial biopsy or D&C for endometrial sampling with cytological or histological examination

- Current approach to diagnose the endometrial cancer: biopsy of endometrium

- Invasive
- Can't be serially repeated
- Side effect-bleeding , infection

Late diagnosis leads to Poor Survival of women with endometrial cancer

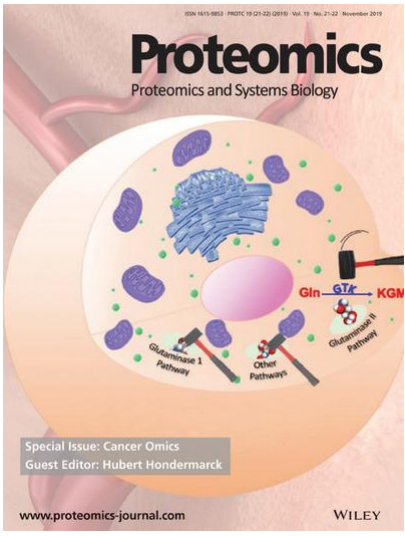


Rationales behind failure to develop promising biomarkers for EC in clinical trials

- Lack suitable systems modeling the complexity of human endometrial cancerous tissue: the current available preclinical models established from cancerous epithelial cells without incorporating the stromal components

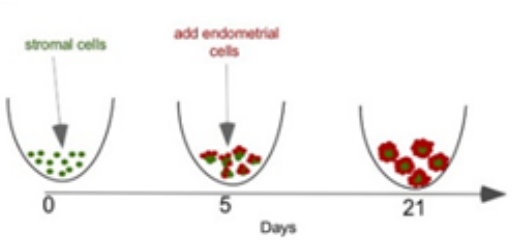
Aim

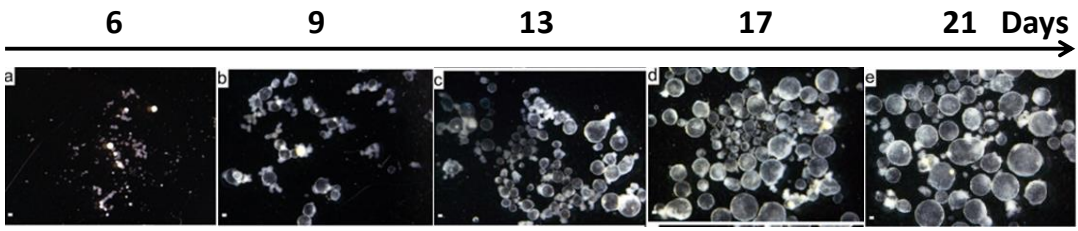
Proteomic analysis of stromal and epithelial cell communications in human endometrial cancer using a unique 3D co-culture model

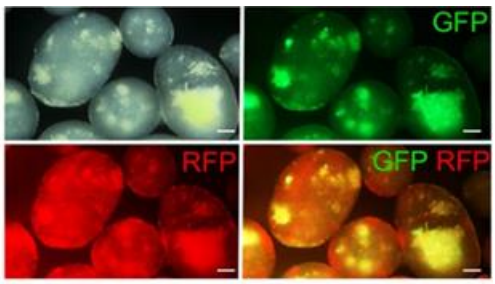


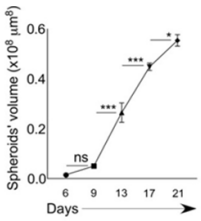
- ❑ Established appropriate system modelling the complexity of human endometrial tissue and disease setting

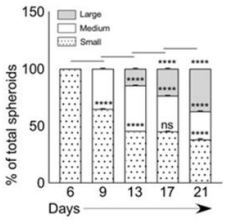
Establishment of a 3D Co-Culture Model with Endometrial Cancer Epithelial Cells with Non-Cancerous Stromal Cells

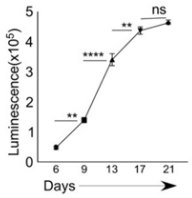






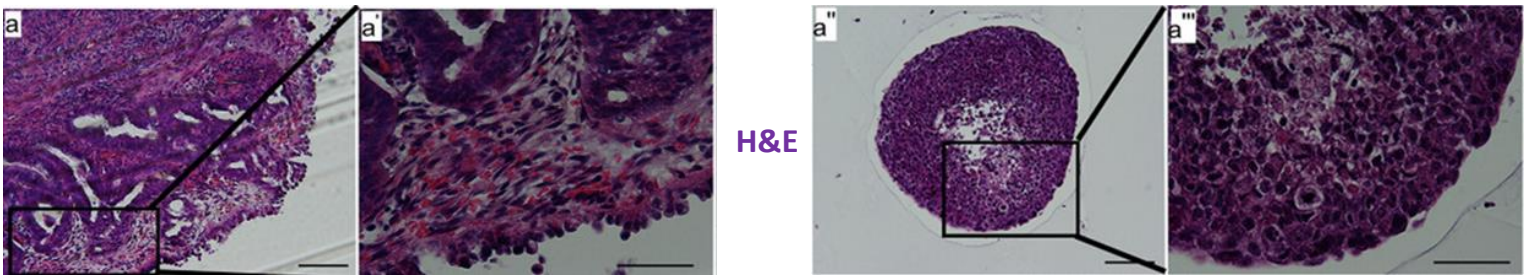




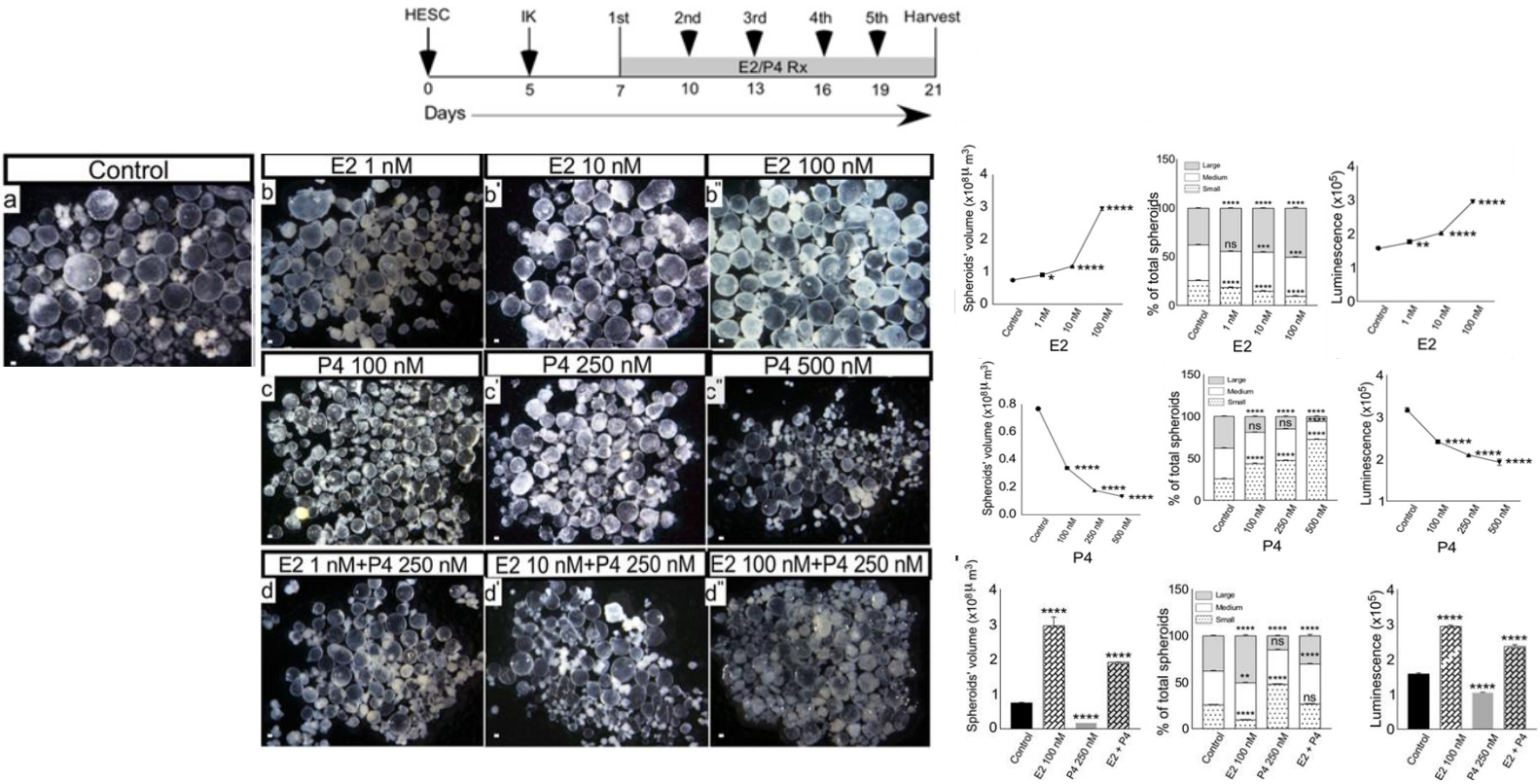


Endometrial Cancer Spheroids Phenocopy the Architecture of Human Endometrial Tissue

Human endometrium | **Co-culture spheroids**



Endometrial Cancer Spheroids Respond to Steroid Hormones



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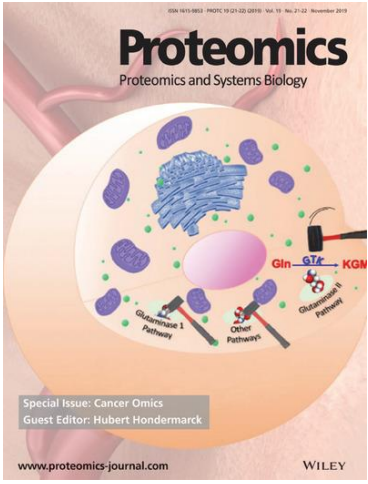


Proteomic analysis of stromal and epithelial cell communications in human endometrial cancer using a unique 3D co-culture model

- ❑ To confirm the similarity of co-culture spheroids and human endometrial cancerous tissue in biological architecture and functional signaling

- ➢ Utilised the more sophisticated, high-throughput technology of omics approaches particularly the proteomic to characterise and quantify the expressed proteins in cancerous co-culture spheroids

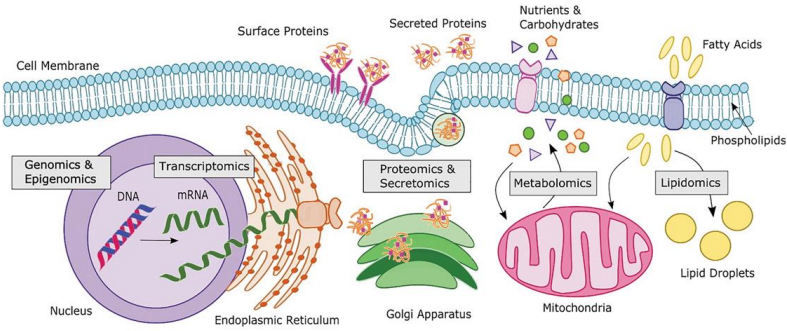
- ➢ Comparison the cancerous co-culture spheroids-based proteome with those getting of patients samples or presence in human database helped in developing many of promising less-invasive, accurate diagnostic biomarkers and targeting therapy for endometrial cancer

Omics Technology

Omics technology: is a more sophisticated, high-throughput techniques decoded the complexity and behaviour of biological systems. They offered a global view of biological processes placed in physiological and pathological situations related to its simultaneous analysis of thousands of molecules in a single biological sample with the aim of predictive disease biomarkers and improving patient management

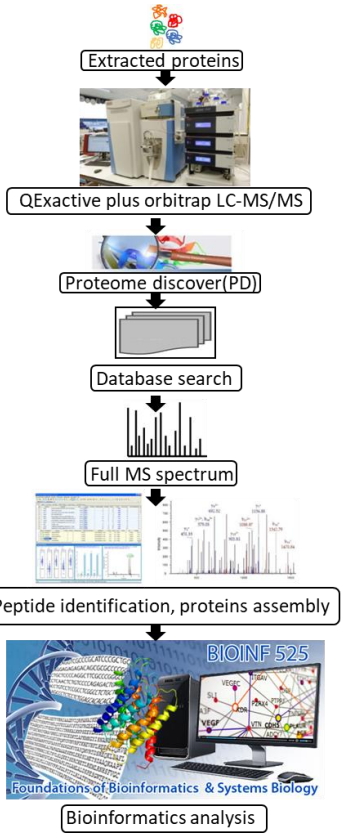
Omics approaches:

- Genomics and epigenomics: analyze nucleic DNA
- Transcriptomics: analyzes all RNA molecules (from messenger RNA in the nuclei to ribosomal RNA in the endoplasmic reticulum)
- Proteomics and secretomics: analyze proteins that synthesized in the endoplasmic reticulum and transported to the membranes or lysosomes or secreted through the golgi apparatus and secretory vesicles
- Metabolomics: analyzes all metabolites produced and consumed by the cell and processed in the mitochondria
- Lipidomics: analyzes all lipid species that are located in membranes and other organelles



Proteomics workflow

- Extraction proteins of human tissues, biological fluids or cells culture
- Lysis the extracted protein with lysis buffer (Na2CO3+PI+PhI)using probe sonication
- Dissolved proteins in urea, reduced (DTT), alkylated (IAA) and digested (Lys-C/trypsin)
- Separation and analysis digested peptide using QExactive plus orbitrap LC-MS/MS
- Acquisition MS data (Xcalibur) then imported into Proteome Discoverer (PD) and searched against the human entries in UniProt
- Identified peptides and list of proteins with strict threshold of FDR less than 1% and contained at least two unique peptides
- Bioinformatics analysis
  - PANTHER/functional analysis
  - IPA/pathway signalling
  - String/Interactome and network modelling

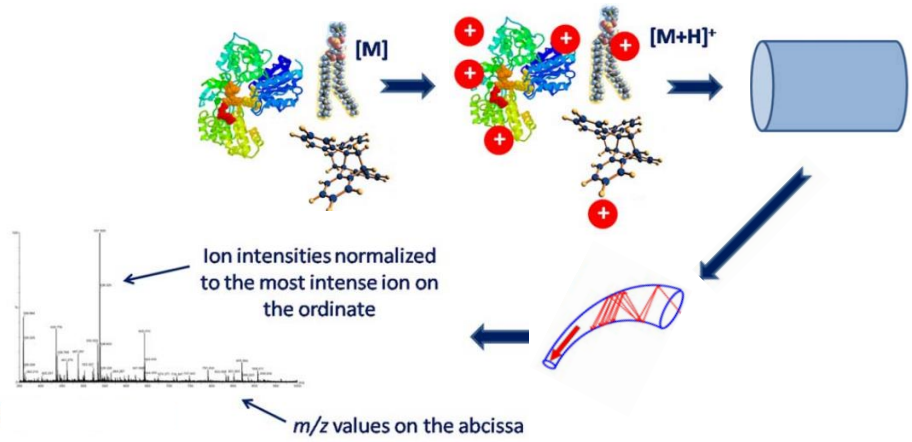


Mass spectrometer

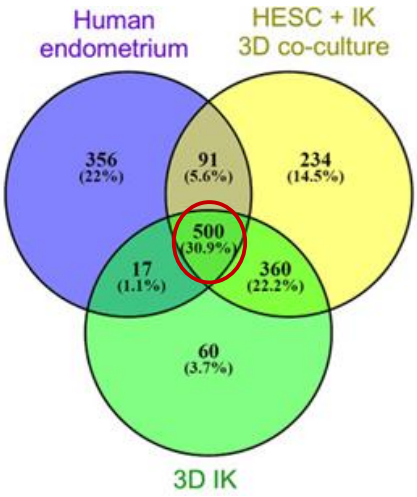
Mass spectrometer is a sensitive technique used to detection and quantification molecules based on their mass to charges (m/z) ratio

Mass spectrometer (MS)

- Ionization source Like ESI
- Mass analyzer where they separate the molecules according to m/z
- Detector which count the ion current and transform into electric pulse that form a peak in the mass spectrum



Proteomic and IPA Analysis of Co-Culture Spheroids are Closely Related to Human Endometrial Cancer and Endometrium in Biological Architecture and Functional Signaling



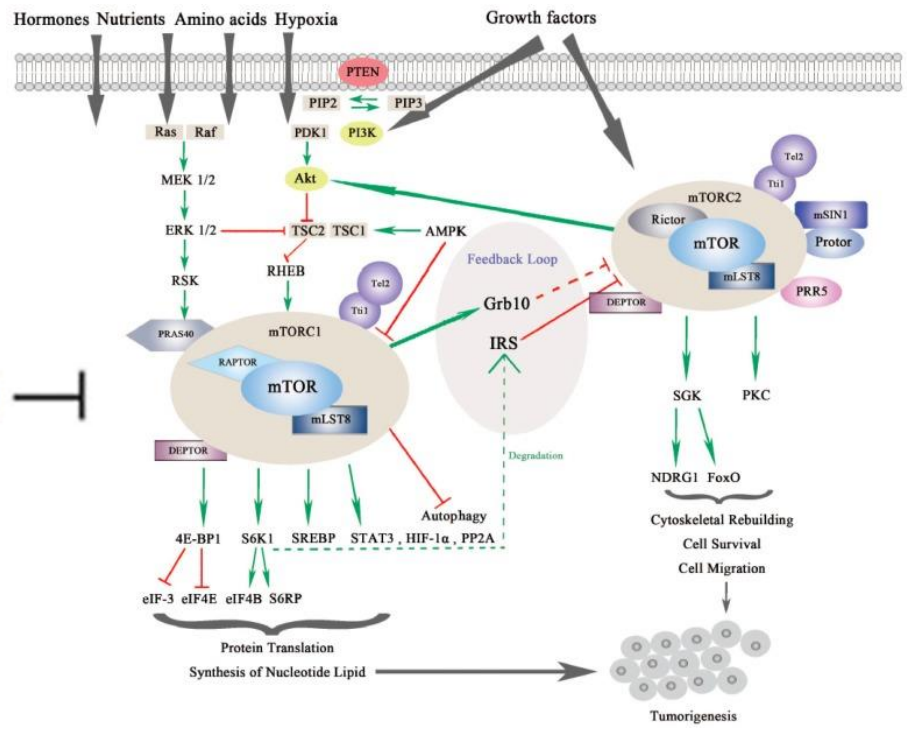
EIF2 signaling	4.99E+01
Regulation of eIF4 and p70S6k signaling	2.04E+01
Protein ubiquitination pathway	1.52E+01
mTOR Signaling	1.45E+01

Everolimus (RAD001) inhibits tumor growth via targeting the mTOR (mammalian target of rapamycin) Signaling

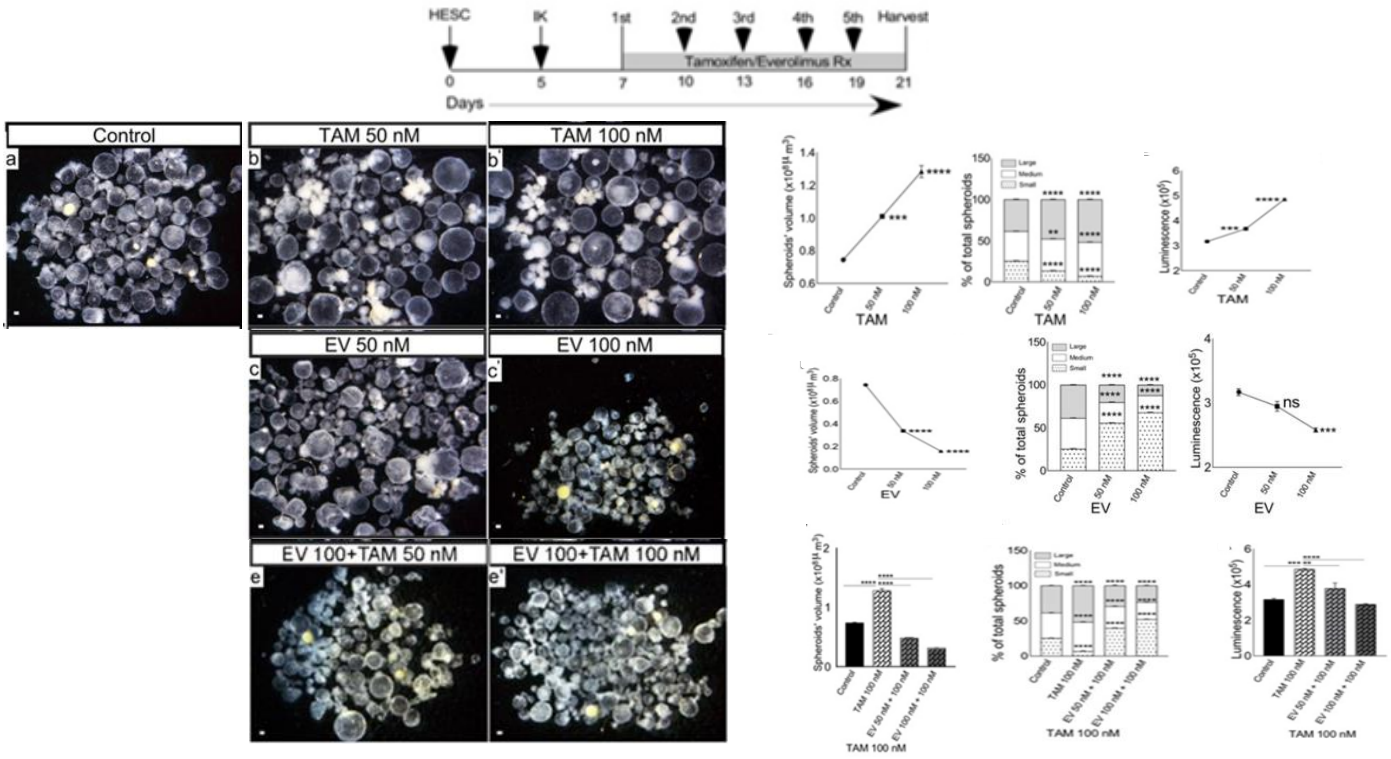
Everolimus (RAD001),

a Rapamycin Derivative,

is a Selective and Orally Active mTOR1 Inhibitor



Treatment with an mTOR Inhibitor (Everolimus) Suppresses the Growth Promoting Effects of Tamoxifen



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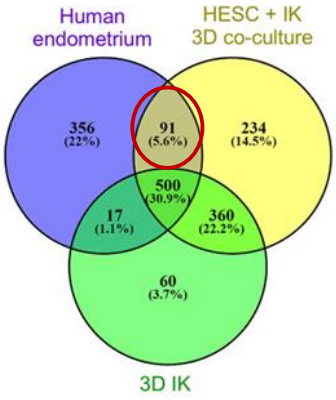
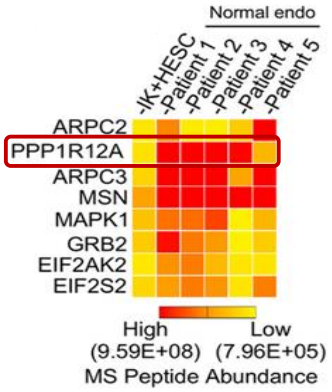
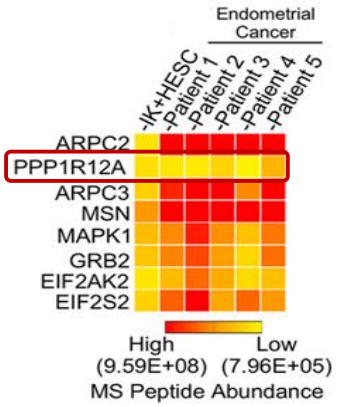


	RhoA	Integrin	Ephrin Receptor	PDGF	VEGF	Insulin Receptor	EGF	ErbB2-ErbB3	ErbB4	BMP
Human endometrium	+	+	+	+	+	+	+	+	+	+
HESC + IK 3D co-culture	+	+	+	+	+	+	+	+	+	+
3D IK	-	-	-	-	-	-	-	-	-	-





Human Protein Atlas Database confirmed the similarity in expression of PPP1R12A (a tumour suppressor protein) among the human endometrial cancerous spheroids and human endometrial tissues

Human Protein Atlas information on PPP1R12A

Normal endometrium tissue
Staining: High
Intensity: Strong
Quantity: 75%-25%
Location: Cytoplasmic/membranous

Endometrial cancer
Staining: Low
Intensity: Moderate
Quantity: <25%
Location: Cytoplasmic/membranous

Endometrial cancer - protein expression. Using specific HPA071956 antibody staining, samples can be filtered based on the following categories: high, medium, low and not detected. The assays and annotation is described by the Human Protein Atlas.

Protein expression summary: Cancer tissues showed cytoplasmic and membranous staining of varying intensities. Endometrial cancer tissue displayed moderate cytoplasmic and membranous staining intensities lower than normal endometrium tissue.

Activation the Rho/ROCK pathway implicated in carcinogenesis through inhibiting the MYPT1

nature COMMUNICATIONS

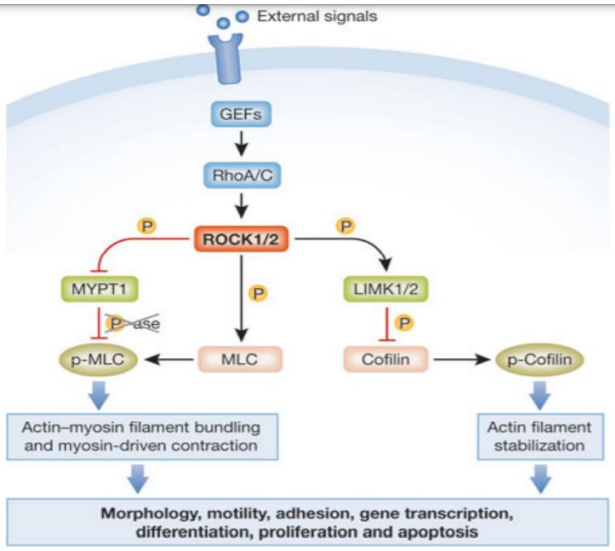
ARTICLE

Received 20 Aug 2016 | Accepted 24 May 2017 | Published 24 Jul 2017

DOI: 10.1038/ncomms16013 OPEN

Actomyosin drives cancer cell nuclear dysmorphia and threatens genome stability

Tohru Takaki[1,2], Marco Montagner[3,*], Murielle P. Serres[1,4,*], Maël Le Berre[5], Matt Russell[6], Lucy Collinson[6], Karoly Szuhai[7], Michael Howell[8], Simon J. Boulton[2], Erik Sahai[3] & Mark Petronczki[1,9]



The nuclear dysmorphia of cancer cells and nuclear shape in general are controlled by the balance of opposing ROCK and PPP1R12A phosphatase activities on myosin regulatory light chain. Hyperactivated and phosphorylated myosin light chain can generate sufficient actomyosin contractility and possibly forces to deform, and even break the nuclear envelope triggering genome instability.

Downregulation expression of PPP1R12A (MYPT1) enhance tumor resistance in ovarian cancer via targeting the hippo pathway and increasing the stemness

IJC
International Journal of Cancer

Expression profiles of PRKG1, SDF2L1 and PPP1R12A are predictive and prognostic factors for therapy response and survival in high-grade serous ovarian cancer

Giuseppe Benvenuto[1], Paola Todeschini[2,3], Lara Paracchini[4], Enrica Calura[1], Robert Fruscio[5], Chiara Romani[2,6], Luca Beltrame[4], Paolo Martini[1], Antonella Ravaggi[2,7], Lorenzo Ceppi[5], Gabriele Sales[1], Federica Donati[4], Patrizia Perego[8], Laura Zanotti[2], Sara Ballabio[4], Tommaso Grassi[5], Martina Delle Marchette[5], Germana Tognon[3], Enrico Sartori[3], Marco Adorni[5], Franco Odicino[3], Maurizio D'Incalci[4], Eliana Bignotti[2,3], Chiara Romualdi[1] and Sergio Marchini[4]

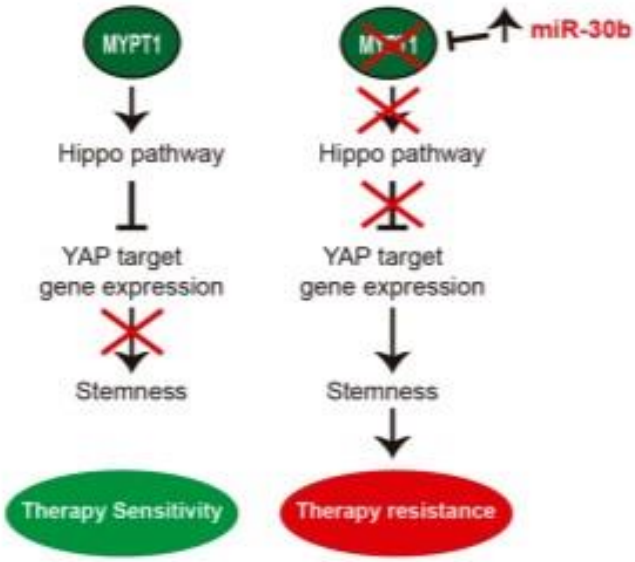
Muñoz-Galván *et al. Molecular Cancer* (2020) 19:7
https://doi.org/10.1186/s12943-020-1130-z

Molecular Cancer

RESEARCH Open Access

Downregulation of *MYPT1* increases tumor resistance in ovarian cancer by targeting the Hippo pathway and increasing the stemness

Sandra Muñoz-Galván[1,2], Blanca Felipe-Abrio[1,2], Eva M. Verdugo-Sivianes[1,2], Marco Perez[1,2], Manuel P. Jiménez-García[1,2], Elisa Suarez-Martinez[1,2], Purificacion Estevez-Garcia[1,2] and Amancio Carnero[1,2*]



Clinical significance

Developing a 3D model reflected the complexity of endometrial tissue and cancer setting by incorporating the stromal cells with cancerous epithelial cells

The proteomics technology confirmed the comparable biological properties and functional signaling of cancerous co-culture spheroids with those seen in patients populations and human database

Utilised cancerous co-culture spheroids in identification the carcinogenic molecular pathways, discovering biomarkers and targeting drugs that help in earlier diagnosis, earlier treatment, prevention the tumor prognosis, and reduced the mortality


Thank You