Effect of KRAS mutation in different colon cancer cell lines

Overview

- Introduction
- Objectives
- Proposed methodology
- Predicted outcomes
- Project timeline
- References

Introduction

Cancer

- In 2014, approximately 0.6 million deaths were due to cancer
- Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells
- Most common cancers worldwide occur in the lung, prostate, breast and colorectum (Siegel et al., 2014)

(Stewart and Wild, 2014)

Genetic basis

- Mutations that activate oncogenes contribute to development of cancer
  - MAPK: mitogen-activated kinase pathway

    KRAS, BRAF, NRAS
  - P13K: phosphatidylinositol 3-kinase pathway

    PIK3CA

(Brenner et al., 2007)
(Ogino et al., 2011)

MAPK signaling pathway

- KRAS gene is a part of MAPK signaling pathway
- Chain of proteins in cell that links extracellular signals to DNA of nucleus controlling fundamental cellular processes
- It regulates cell proliferation, differentiation and apoptosis
- It involves activation of several membranal signaling molecules followed by a sequential stimulation of several cytoplasm protein kinases
- When one of proteins in MAPK pathway is mutated, it can remain ‘on’ or ‘off’ causing uncontrolled cell growth that lead to development of cancer

(Dhillon et al., 2007)

KRAS gene

- Kirsten rat sarcoma viral oncogene homolog (KRAS)
- It is one of the most activated oncogenes and acts as a molecular on and off switch
- It encodes for KRAS protein which is a GDP/GTP-binding protein that act as intracellular signal transducers
- KRAS gene is involved in cytokine signaling, cell adhesion, cell survival, proliferation, apoptosis, and colon development

(Oliveira, 2004)

KRAS mutation

- Found in most of the cancers
- Present in 36%-40% colon cancer patients
- Often detected in codon 12 and 13 of chromosome 12
- Mutations in KRAS gene are common in adenomas
- When mutated, KRAS gene remains switched on all the time stimulating cells to grow and divide abnormally forming a tumor

(Wilson et al., 2010)

KRAS mutation

Drug resistance

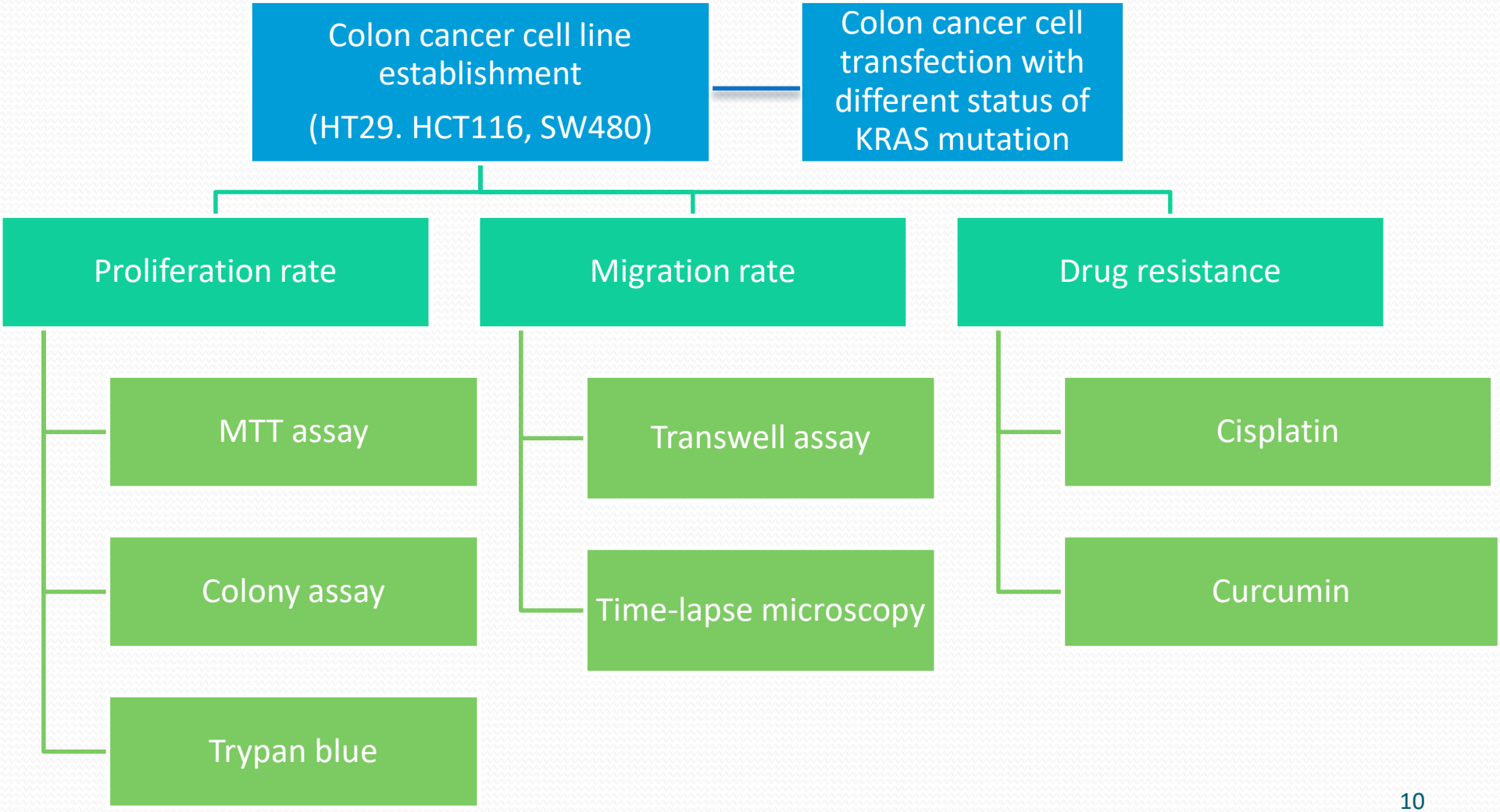
- Research suggests that tumors with KRAS mutation treated using cisplatin (chemotherapy drug) leads to emergence of resistant tumors with increased repair ability
- Initial responsiveness to cisplatin is high but the majority of cancer patients will eventually relapse with cisplatin-resistant disease
- Also, Curcumin which is an active ingredient of turmeric possessing anti-inflammatory and anti-cancer properties has shown to inhibit cell growth of HT-29 cells in a concentration- and time-dependent manner
- As therapy for cancer becomes more effective, acquired resistance becomes more common as well

(Garassino et al., 2010)
(Goel, Boland and Chauhan, 2001)

Objectives

1. To determine how KRAS mutation affects proliferation rate of colon cancer cells
2. To determine how migration rate of colon cancer cells are affected by KRAS mutation
3. To determine whether colon cancer cells with KRAS mutation affect sensitivity to therapeutic agents

Proposed methodology



Colon cancer cell line establishment

	HT29	HCT116	SW480
Organism	Homo sapiens, human		
Tissue	Colon		
Morphology	Epithelial		
Disease	colorectal adenocarcinoma	colorectal carcinoma	colorectal adenocarcinoma
KRAS status	Wild type KRAS	Mutation in codon 13 of RAS gene	Mutation in codon 12 of RAS gene

(ATCC, 2015)

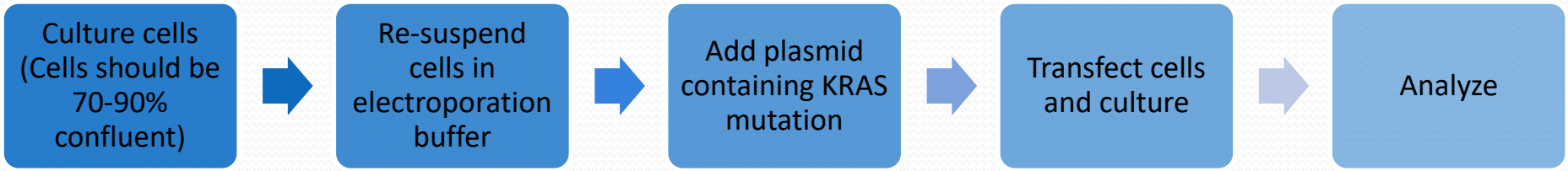
Experimental design

1. Conducting proliferation, migration and drug resistance assays for HT29, HCT116 and SW480 colon cancer cell lines and comparing results

2. Conducting proliferation, migration and drug resistance assays for HT29 cell line with and without KRAS mutation transfection and comparing results

Transfection of HT29 with KRAS mutation

- Transfection is the introduction of foreign DNA in to nucleus of eukaryotic cells
- Purpose : Transfecting HT29 cells with KRAS mutation and comparing proliferation rate, migration rate and drug resistance of HT29 cells with and without KRAS mutation



Objective 1: Determine the effect of KRAS mutation on proliferation rate of colon cancer cells

- MTT cell viability assay
  - 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay
  - Colorimetric assay
  - Based on conversion of MTT to formazan crystals by mitochondria in living cells
  - Total mitochondrial activity is related to number of viable cells

(van Meerloo et al., 2011)
(Franken et al., 2006)

Colony formation assay

- *In-vitro* cell survival assay
- Based on ability of single cell to grow into a colony
- Colonies are fixed with glutaraldehyde, stained with crystal violet and counted using the microscope

Trypan blue assay

- Dye exclusion procedure
- Trypan blue is a stain used to colour dead cells blue
- Stained solution is loaded to hemacytometer and counted with a microscope

(Katz et al., 2008)

Objective 2: Determine how migration rate of colon cancer cells are affected by KRAS mutation

Transwell migration assay

- This assay allows motility of cancer cells to be studied
- Cells are placed on upper layer of a micro porous membrane
- Following incubation, cells that have migrated the membrane are stained and are quantified by fixing and counting

(Mandelboim, 2006)

Time-lapse microscopy

- Microscopic image sequences are recorded and viewed at a greater speed
- Provides accelerated view of microscopic process

(Chen, Zhou and Wong, 2006)

Objective 3: To determine whether colon cancer cells with KRAS mutation affect sensitivity to therapeutic agents

- Anti cancer drug resistance will be assessed by measuring IC_{50} of colon cancer cell lines to Cisplatin and Curcumin
- Phenotypic resistance assays measure susceptibility of cells to anti cancer drugs in terms of concentration of drug required to inhibit biological activity *in vitro* by defined amount as 50% (IC_{50})
- IC_{50} will be determined by constructing a dose-responsive curve with the MTT assay data

Predicted outcomes

It is expected that,

- KRAS mutation increases proliferation rate and migration rate of colon cancer cells
- KRAS mutation increases anti-cancer drug resistance in colon cancer cells

Project timeline

	March	April	May	June	July	August	September	October
Proposal presentation								
Literature review								
Cell line establishment								
Transfection with KRAS mutated plasmid								
Proliferation, migration and drug resistance assays								
Time-lapse microscopy								
Thesis writing								

References

- ATCC, (2015). *HCT 116 ATCC Â® CCL-247â„¢ Homo sapiens colon colorectal carcin*. [online] Atcc.org. Available at: http://www.atcc.org/products/all/CCL-247.aspx [Accessed 9 Apr. 2015].
- ATCC, (2015). *HT-29 ATCC Â® HTB-38â„¢ Homo sapiens colon colorectal adenocar*. [online] Atcc.org. Available at: http://www.atcc.org/products/all/HTB-38.aspx [Accessed 9 Apr. 2015].
- ATCC, (2015). *SW480 [SW-480] ATCC Â® CCL-228â„¢ Homo sapiens colon Dukes' typ*. [online] Atcc.org. Available at: http://www.atcc.org/products/all/CCL-228.aspx [Accessed 9 Apr. 2015].
- Boyle, P. and Levin, B. (2008). *World cancer report*. Lyon: IARC Press.
- Brenner, H., Hoffmeister, M., Stegmaier, C., Brenner, G., Altenhofen, L. and Haug, U. (2007). Risk of progression of advanced adenomas to colorectal cancer by age and sex: estimates based on 840 149 screening colonoscopies. *Gut*, 56(11), pp.1585-1589.
- Chen, X., Zhou, X. and Wong, S. (2006). Automated Segmentation, Classification, and Tracking of Cancer Cell Nuclei in Time-Lapse Microscopy. *IEEE Transactions on Biomedical Engineering*, 53(4), pp.762-766.
- Dhillon, A., Hagan, S., Rath, O. and Kolch, W. (2007). MAP kinase signalling pathways in cancer. *Oncogene*, 26(22), pp.3279-3290.
- Franken, N., Rodermond, H., Stap, J., Haveman, J. and van Bree, C. (2006). Clonogenic assay of cells in vitro. *Nature Protocols*, 1(5), pp.2315-2319.
- Garassino, M., Marabese, M., Rusconi, P., Rulli, E., Martelli, O., Farina, G., Scanni, A. and Broggini, M. (2010). Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer. *Annals of Oncology*, 22(1), pp.235-237.
- Gazdar, A., Gao, B. and Minna, J. (2010). Lung cancer cell lines: Useless artifacts or invaluable tools for medical science?. *Lung Cancer*, 68(3), pp.309-318.
- Goel, A., Boland, C. and Chauhan, D. (2001). Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. *Cancer Letters*, 172(2), pp.111-118.
- Katz, D., Ito, E., Lau, K., Mocanu, J., Bastianutto, C., Schimmer, A. and Liu, F. (2008). Increased efficiency for performing colony formation assays in 96-well plates: novel applications to combination therapies and high-throughput screening. *BioTechniques*, 44(2), p.Pix-Pxiv.
- Lim, G., Rampal, S. and Halimah Yahaya., (2008). *Cancer incidence in Peninsular Malaysia, 2003-2005*. Kuala Lumpur: National Cancer Registry.
- Mandelboim, O. (2006). Trans-well migration assay. *Protocol Exchange*.
- Odd Organisms, (2015). [image].
- Ogino, S., Shima, K., Meyerhardt, J., McCleary, N., Ng, K., Hollis, D., Saltz, L., Mayer, R., Schaefer, P., Whittom, R., Hantel, A., Benson, A., Spiegelman, D., Goldberg, R., Bertagnolli, M. and Fuchs, C. (2011). Predictive and Prognostic Roles of BRAF Mutation in Stage III Colon Cancer: Results from Intergroup Trial CALGB 89803. *Clinical Cancer Research*, 18(3), pp.890-900.
- Oliveira, C. (2004). Distinct patterns of KRAS mutations in colorectal carcinomas according to germline mismatch repair defects and hMLH1 methylation status. *Human Molecular Genetics*, 13(19), pp.2303-2311.
- Siegel, R., Ma, J., Zou, Z. and Jemal, A. (2014). Cancer statistics, 2014. *CA: A Cancer Journal for Clinicians*, 64(1), pp.9-29.
- Stewart, B. and Wild, C. (2014). *World cancer report 2014*.
- van Meerloo, J., Kaspers, G. and Cloos, J. (2011). Cell Sensitivity Assays: The MTT Assay. *Methods in Molecular Biology*, pp.237-245.
- Wilson, P., LaBonte, M. and Lenz, H. (2010). Molecular Markers in the Treatment of Metastatic Colorectal Cancer. *The Cancer Journal*, 16(3), pp.262-272.
- YEH, R. (2004). NO-donating nonsteroidal antiinflammatory drugs (NSAIDs) inhibit colon cancer cell growth more potently than traditional NSAIDs: a general pharmacological property?. *Biochemical Pharmacology*, 67(12), pp.2197-2205.

Thank you