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BCL-2, HER1, HER2, HER3 AND HER4 EXPRESSION IN PRIMARY COLORECTAL ADENOCARCINOMA

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chairman : Associate Professor Norhafizah Mohtarrudin, MBBS, MPath, AM
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In Malaysia, CRC is the most frequently reported cancer among males and second in females with the prevalence of 16.4% and 10.7%, respectively. Most cases occur sporadically, only 5% of the cases are inherited. There is a 50% risk of recurrence for the CRC survivor. Hence biomarkers to improve the prognosis, treatment outcomes and risk assessment are in dire need. The potential biomarkers include BCL-2 and HERs family members (HER1, HER2, HER3, and HER4). BCL-2 is an anti-apoptotic protein and its expression has been associated with tumour grading, staging, and prognosis. It affects tumour genesis by inhibiting cell apoptosis. Presence of BCL-2 expression enhances cancer survival rate. HERs family members are known as a surface receptor. Its activation occurs via ligands binding, leading to downstream signalling that influences cell proliferation. Mutations in HERs family members lead to activation of a series of signalling cascade which has numerous effects on protein expression. HER1, HER2, HER3 and HER4 aberrations in signalling contributes to malignancies, gene amplification and enhanced transcription in the CRC. This study aims to determine the protein expression of BCL-2 and HERs family members in CRC cases, using immunohistochemistry (IHC) technique. A total of 94 **FFPE** samples were collected from the Hospital Serdang archives from year 2008 until year 2015. Data on demographic and clinicopathologic were retrieved from the database. Protein expressions were detected in the cytoplasm, membranous, or/and nucleus region. The expressions were scored with semi-quantitative scoring system and graded as 0 (absent), 1+ (weak), 2+ (moderate), or 3+ (strong). Association test was used to determine the *p* - value ($p < 0.05$). BCL-2 expression was seen immunopositive in 26.6% cases. HERs family members showed immunopositivity in 31.9%, 13.8%, 42.6%, and 31.9% in HER1, HER2, HER3, and HER4 respectively. There was a significant association between HER1 and HER3 expression ($p < 0.001$), HER1 and HER4 expression ($p = 0.019$) and between HER3 and HER4 expression ($p = 0.019$). Significant association was seen between BCL-2 and HER1 expression ($p = 0.022$). HER1/HER3 co-expression was significantly associated with tumour grading ($p = 0.030$) and HER3/HER4 co-expression was significantly related with lymph node metastasis ($p = 0.018$). HER1 expression was

associated with age ($p = 0.018$), Malay ethnicity ($p = 0.038$), tumour grading ($p = 0.011$), and diabetes mellitus ($p = 0.044$). A significant association was seen between HER2 and diabetes mellitus ($p = 0.024$). HER3 expression was significantly associated with gender ($p = 0.027$), tumour grading ($p = 0.023$), lymph nodes metastasis ($p = 0.049$), and smoking ($p= 0.033$). Likewise, HER4 expression was significantly associated with ethnicity ($p < 0.001$) and tumour grading ($p = 0.021$). This study shows that BCL2 and the HERs family members are overexpressed in CRC. The implications of these results for the CRC biomarkers are that, generally, overexpression of HERs family members are more likely inclined towards poor prognosis. While BCL-2 failed to show any relationship with demographic and clinicopathologic parameters. Results in co-expression among antibodies indicated relation and dependency with each other through downstream of pathways. BCL-2, HER1, HER2, HER3 and HER4 could be further developed for targeted therapy receptors and prognostic biomarkers for CRC.

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BCL-2, HER1, HER2, HER3 DAN HER4 EKSPRESI DALAM KOLOREKTAL ADENOKARSINOMA PRIMER

Oleh

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Di Malaysia, kolorektal kanser adenokarsinoma (CRC) merupakan kanser yang paling kerap dilaporkan di kalangan lelaki dan kedua di kalangan wanita dengan statistik masing-masing adalah 16.4% dan 10.7%. Kebanyakan kes yang berlaku adalah secara rawak dan hanya 5% adalah kes yang diwarisi secara genetik. Terdapat 50% risiko keberulang-laku bagi CRC pemandiri. Justeru itu penanda-penanda bio untuk menambah baiki prognosis, hasil rawatan dan penilaian risiko sangat diperlukan. Penanda aras berpotensi yang dikenal pasti termasuklah BCL-2 dan dari ahli keluarga HER (HER1, HER2, HER3, dan HER4). BCL-2 adalah dari protein *anti-apoptosis* dan ekspresinya berkait rapat dengan gred tumor, peringkat tumor dan prognosis. Ia menjejaskan genetik tumor dengan menghalang sel-sel *apoptosis*. Ekspresi BCL-2 meningkatkan kadar kelangsungan sel-sel kanser. Ahli keluarga HER dikenali sebagai reseptor permukaan. Pengaktifannya terjadi melalui ligan-ligan mengikat, yang membawa kepada isyarat hiliran yang mempengaruhi proliferasi sel. Mutasi di kalangan ahli keluarga HER membawa kepada pengaktifan satu siri lata isyarat yang mempunyai banyak kesan terhadap ekspresi protein. Penyimpangan isyarat HER1, HER2, HER3 dan HER4 menyumbang kepada kejadian kanser, amplifikasi gen dan peningkatan transkripsi dalam CRC. Kajian ini dilakukan untuk menentukan ekspresi protein BCL-2 dan ahli keluarga HER dalam kes-kes CRC dengan menggunakan teknik imunohistokimia (IHC). Sejumlah 94 sampel tisu yang diproses dalam bentuk tisu terendam formalin-parafin (FFPE) telah dikumpulkan dari arkib Hospital Serdang diantara tahun 2008 hingga tahun 2015. Data mengenai demografi dan klinikopatologi diambil daripada pangkalan data. Ekspresi protein dikesan pada kawasan sitoplasma, membran atau/dan nukleus. Ekspresi diberi skor berdasarkan sistem pemarkahan semi-kuantitatif dan dinilai sebagai 0 (tidak hadir), 1+ (lemah), 2+ (sederhana), atau 3+ (kuat). Ujian pertalian digunakan untuk menentukan nilai p ($p < 0.05$). Terdapat 26.6% kes imunopositif untuk BCL-2. Ahli keluarga HER menunjukkan imunopositifiti sebanyak 31.9% (HER1), 13.8% (HER2), 42.6% (HER3) dan 31.9% (HER4). Terdapat persamaan yang signifikan antara ekspresi HER1 dan HER3 ($p < 0.001$), ekspresi HER1 dan HER4 ($p = 0.019$) dan antara ekspresi HER3 dan HER4 ($p = 0.019$). Persamaan yang signifikan dilihat antara ekspresi BCL-2

dan HER1 (p = 0.022). HER1 / HER3 ekspresi bersama dikaitkan dengan peringkat tumor (p = 0.030) manakala ekspresi bersama HER3 / HER4 berkaitan dengan metastasis nodus limfa (p = 0.018). Ekspresi HER1 dikaitkan dengan umur (p = 0.018), etnik Melayu (p = 0.038), gred tumor (p = 0.011) dan kencing manis (p = 0.044). Pertalian yang ketara dilihat antara HER2 dan kencing manis (p = 0.024). Ekspresi HER3 secara signifikan dikaitkan dengan jantina (p = 0.027), gred tumor (p = 0.023), metastasis nodus limfa (p = 0.049) dan merokok (p = 0.033). Selain itu, ekspresi HER4 berkait dengan etnik (p < 0.001) dan gred tumor (p = 0.021). Kajian ini menunjukkan bahawa ekspresi BCL2 dan ahli keluarga HER wujud dalam CRC. Implikasi keputusan penanda-bio ini terhadap CRC adalah, secara umumnya, ekspresi ahli keluarga HER lebih cenderung kepada prognosis yang buruk. Walau bagaimanapun penanda BCL-2 gagal menunjukkan sebarang hubungkait dengan parameter demografi dan klinikopatologi. Ekspresi bersama antara antibodi menunjukkan hubungan dan pergantungan antara satu sama lain melalui tapak jalan. BCL-2, HER1, HER2, HER3 dan HER4 boleh dikaji dengan lebih mendalam sebagai reseptor sasaran terapi dan penanda bio prognostik untuk CRC.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
μ_C	Proportion Of Unexposed With Outcome
µm	Micrometre
μ_T	Proportion Of Exposed With Outcome
5-FU	5-Fluorouracil
ABC	Avidin-Biotin-Peroxidase Complex
AKT	Protein Kinase B
APAAP	Alkaline Phosphatase-Anti Alkaline Phosphatase
ATP	Adenosine Triphosphate
BAX	Bcl-2 Associated X Protein
BCL-2	B-Cell Lymphoma 2
CEA	Carcinoembryonic Antigen
cm	Centimetre
CRC	Colorectal Cancer
CT-scan	Computed Tomography Scan
DAB	3,3'-Diaminobenzene
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
ECD	Extracellular Domain
ELISA	Enzyme-Linked Immunosorbent Assay
ErbB	Avian Erythroblastosis Oncogene B
ERK	Extracellular Signal-Regulated Kinase
ERRP	EGFR-Related Protein

FDA	Food and Drug Administration
FFPE	Formalin Fixed Paraffin Embedded
FOBT	Faecal Occult Blood Testing
GIT	Gastrointestinal Tract
HB-EGF	Heparin Binding Epidermal Growth Factor Like Growth
HER	Human Epidermal Growth Factor Receptor
HER1	Human Epidermal Growth Factor Receptor-1
HER2	Human Epidermal Growth Factor Receptor-2
HER3	Human Epidermal Growth Factor Receptor-3
HER4	Human Epidermal Growth Factor Receptor-4
ICD	Intracellular Domain
IHC	Immunohistochemistry
JM	Juxtamembrane
kDa	Kilo Dalton
LN	Lymph Node
mAbs	Monoclonal Antibodies
MAPK	Mitogen Activated Protein Kinase
MEK	MAPK/ERK Kinase
MRI	Magnetic Resonance Imaging
NRG	Neuregulin
OS	Overall Survival
PAP	Peroxide-Antiperoxidase
pH	Potential of Hydrogen
PI3K	Phosphatidylinositol 3-Kinase

RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RT	Room temperature
SH2	Src homology 2
SH3	Src homology 3
TKI	Tyrosine Kinase Inhibitors
TKR	Tyrosine Kinase Receptor
TM	Transmembrane Domain
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Study Background

Colorectal cancer (CRC) is an asymptomatic disease in its early development, and generally takes up five to ten years to be obvious. In the United Kingdom (UK), CRC is the second highest cause of death by cancer with 15,000 cases in 2014 (Cancer Research UK). The National Institute of Health (NIH) reported the United State spent more than $216.6 billion for cancer management in 2009 alone. Globally, CRC is the third leading cause of cancer death (Garza-Treviño *et al*., 2015) and is the third most frequently diagnosed cancer in both men and women (Sabounchi *et al*., 2012). Among all the cancer survivors, there is an alarming risk of 50% relapse and it is difficult to achieve a total cancer-free condition (Bandrés *et al*., 2007; Labianca *et al*., 2010). The increasing incidence of CRC has been reported in Asian region, although it was previously considered as a low risk region (Labianca *et al*., 2010). According to the Malaysian National Cancer Registry Report 2007-2011 (2016), CRC is the most common cancer in male Malaysian population and second in female population. Cumulatively, CRC is the second most reported cancer with 13,693 cases between the year 2007 to 2011 (Azizah *et al*., 2016).

Management of CRC depends on the grade and stage of the disease. Currently, surgery is the gold standard for CRC treatment, common for stage II and stage III. However, the routine chemotherapy cocktail use in CRC does not only eradicate cancerous cells, it also indirectly weaken nearby healthy cells (Caley & Jones, 2012). Antibody targeted therapy which selectively kills cancer cells has lesser side effects and could enhance patients recovery rate (Sudhakar, 2009). Molecular markers have been correlated with CRC prognosis and therapeutics approaches, in addition for use in risk assessment. Efficient biomarkers are needed for prognostication and measurement of competency of therapeutic regimens. The ability of cancer cells to avoid apoptosis and increase proliferation rate leads to cell cycle aberration. Concurrently, analysing two related biomarkers brings more information with the potential to serve as dual targeted antibodies (Newton *et al*., 2012; Reimers *et al*., 2013).

The B-cell lymphoma-2 (BCL-2) is a member of the anti-apoptotic proteins which are composed of more than 25 members. BCL-2 is overexpressed in various types of cancers including breast, ovaries, thyroid and colon. A previous in vitro study done on colorectal cell lines (CaCo2, Colo205, HT29 and SW480) demonstrated that BCL-2 was significantly overexpressed. Knockdown of BCL-2 hindered invasiveness and motility in colorectal cell lines, while overexpression of BCL-2 enhances cell migration in two-fold (Koehler *et al*., 2013). Several studies have also indicated that BCL-2 expression is significantly associated with better prognosis in long term and improved survival (Han *et al*., 2006; Kouraklis *et al*., 2003; Meterissian *et al*., 2001).

Multiple studies have suggested that the heterodimerization of HER family members plays a crucial role in tumourigenesis and in the development of resistance to therapy in CRC cases. HER or ErbB family members have been extensively studied in different types of cancer including breast, lung, skin and colon cancer for biomarkers purposes. Numerous studies have indicated that HER1, HER2, and HER3 properties are significantly associated with CRC conditions. The role of HER4 is still understudied, although its involvement in CRC carcinogenesis seems likely. HER1 expression has been found to be related to TNM stage III. Subsequently, HER1 knockdown interfered with SW480 colon cancer cells anchorage-independent cell growth ability (Li *et al*., 2014; Spano *et al*., 2005). Recent studies have suggested that HER2 activation correlated with lymph node and tumour differentiation (Half *et al*., 2004; Kountourakis *et al*., 2006). Further study in colon cancer cell line indicated that HER2 overwhelming signals increase cancer metastasis activity and lead to poor clinical outcome (Yu & Hung, 2000). An earlier study in gastric cancer determined that HER3 overexpression is strongly associated with advanced tumour progression and is a poor prognostic indicator (Hayashi *et al*., 2008). Another study in breast cancer revealed connection between HER3 overexpression and reduced survival rate (Witton *et al*., 2003).

HER4 cell lines study indicated that the disruption of HER4 signalling in poorly differentiated CRC cell lines inhibits cell survival mechanism, but HER4 overexpression enhance cell survival and growth. Excessive HER4 signalling is also documented as a factor for epithelial inflammation (Williams *et al*., 2015). Recent studies have established that HER family members can interact and bond with each other to form an array of heterodimer complexes. Therefore, the activation of one receptor could modulate the activity of other HER family members. Co-expression could extend the range of downstream signalling events. Their interaction among each other might enable cells to specifically react to exogenous stimuli. Co-expression of HER2 and HER4 had a shorter overall survival in late stage and poor prognosis CRC cases (Lee *et al*., 2002). A better survival in bladder cancer was found in HER3 and HER4 co-expression (Memon *et al*., 2004). Co-expression of HER2 and HER3 also were highly associated in breast cancer (Witton *et al*., 2003).

1.2 Problem statement

CRC is one of the most common cancers with high recurrence and mortality rates. Currently, biomarkers available for CRC are less sensitive and specific for prognostic and therapeutic purposes. Treatment resistance is also an issue in CRC. One of the factors contributing to treatment resistance and relapse issues is the aberrant apoptotic signalling. High anti-apoptotic signals from BCL-2, for example, can reduce chemotherapy efficiency and enhance cancer cell survival. This situation is likely to cause poor prognosis and relapse in patients. Targeted cancer therapy promises less side effects and better quality of life. Blockage of HER family members contributes to the decrease of cancer cell activities including delaying tumour growth, inducing tumour shrinkage as well as reducing metastasis rate. Correlation of the molecular alterations with demographic and clinicopathologic data could also provide important information on the aetiology of CRC.

1.3 Objectives

General objective:

To determine protein expression of BCL-2, HER1, HER2, HER3, and HER4 in CRC patients using immunohistochemistry (IHC) technique.

Specific objectives:

1. To determine the demographic and clinicopathologic distribution of CRC cases.
2. To determine the distribution of BCL-2 and HERs (HER1, HER2, HER3, and HER4) protein expression in CRC cases.
3. To determine the association among HERs (HER1 and HER2, HER1 and HER3, HER1 and HER4, HER2 and HER3, HER2 and HER4, and HER3 and HER4) expression in CRC cases.
4. To determine the association between BCL-2 and HERs (BCL-2 and HER1, BCL-2 and HER2, BCL-2 and HER3, and BCL-2 and HER4) expression in CRC cases.
5. To determine the association of BCL-2 and HERs (HER1, HER2, HER3, and HER4) protein expression with the demographic and clinicopathologic parameters.

1.4 Research hypothesis

1. BCL-2, HER1, HER2, HER3, and HER4 overexpression are detected in CRC cases.
2. There are significant associations among HERs (HER1 and HER2, HER1 and HER3, HER1 and HER4, HER2 and HER3, HER2 and HER4, and HER3 and HER4) expression in CRC cases.
3. There are significant associations between BCL-2 and HERs (BCL-2 and HER1, BCL-2 and HER2, BCL-2 and HER3, and BCL-2 and HER4) expression in CRC cases.
4. There are significant associations of BCL-2 and HERs (HER1, HER2, HER3, and HER4) protein expression with the demographic and clinicopathologic parameters.

1.5 Significance of study

1. This study provides baseline data on CRC cases in relations to BCL-2, HER1, HER2, HER3, and HER4 protein expressions.

2. Findings of this study could suggest the potential use of biomarkers for therapeutic purposes such as in BCL-2 (Venetoclax, (Cang *et al*., 2015)), HER1 (Cetuximab (Pozzi *et al*., 2016)), and HER2 (Neratinib (Kavuri *et al*., 2015)). Including in some dual targeted therapy drugs in HER1/HER3 (MEHD7945A (Huang *et al*., 2013)) and HER1/HER2 (Lapatinib (Kim *et al*., 2009)).

3. BCL-2, HER1, HER2, HER3, and HER4 antibodies could be used as IHC biomarkers to determine suitable patients with proper therapeutic approaches.

1.6 Research conceptual framework

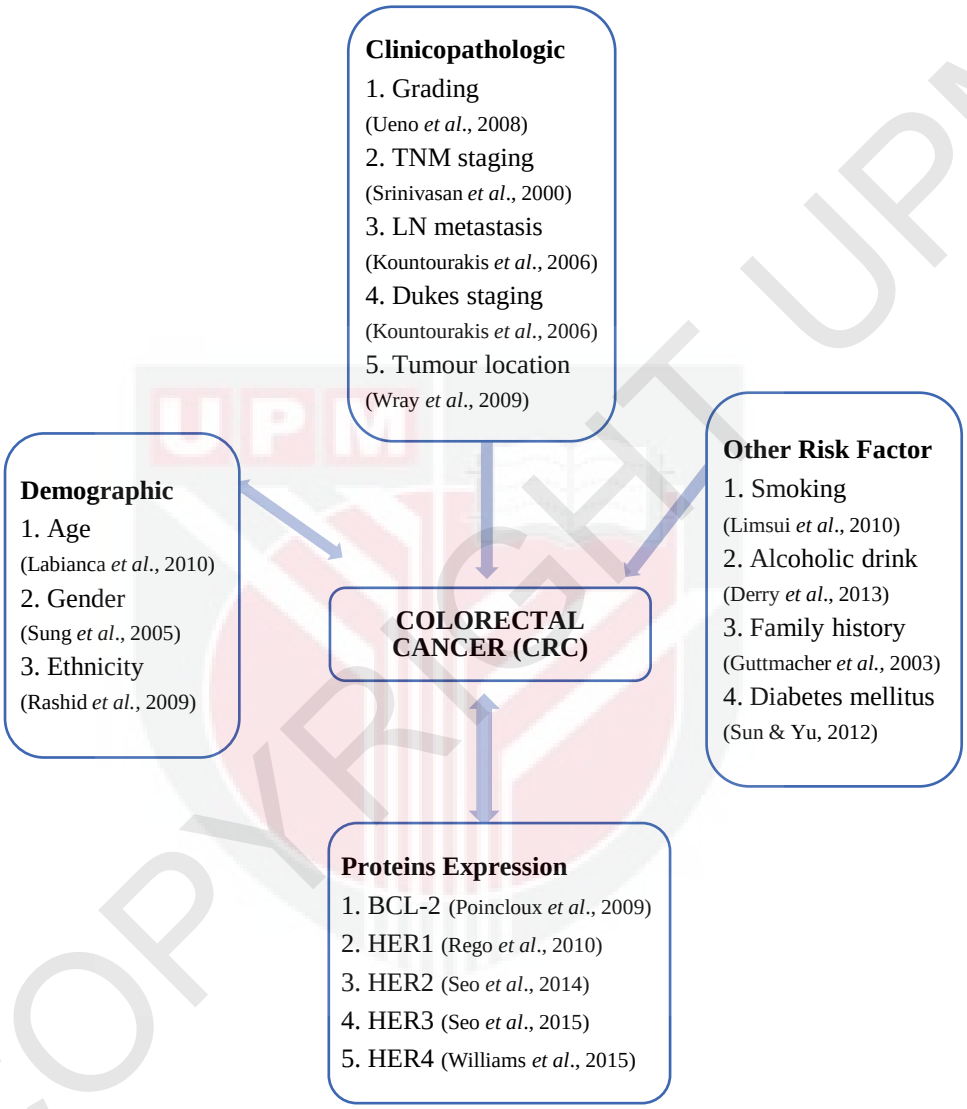

Figure 1.1 : Variables associated with CRC cases

REFERENCES

Alireza, A., Raheleh, S., Abbass, R., Mojgan, M., Mohamadreza, M., Gholamreza, M., & Shadi, B. (2008). An immunohistochemistry study of tissue bcl-2 expression and its serum levels in breast cancer patients. *Ann. N.Y. Acad. Sci.*, *1138*, 114–20.

Amin, D. N., Campbell, M. R., & Moasser, M. M. (2010). The role of HER3, the unpretentious member of the HER family, in cancer biology and cancer therapeutics. *Seminars in Cell & Developmental Biology*, *21*(9), 944–50.

Amin, M. M., Asaad, G. F., Salam, R. M. A., El-abhar, H. S., & Arbid, M. S. (2014). Novel CoQ10 Antidiabetic Mechanisms Underlie Its Positive Effect : Modulation of Insulin and Adiponectine Receptors , Tyrosine Kinase , PI3K , Glucose Transporters , sRAGE and Visfatin in Insulin Resistant / Diabetic Rats. *PloS One*, *9*(2), 1–12 (e89169).

Aqeilan, R. I., Donati, V., Gaudio, E., Nicoloso, M. S., Sundvall, M., Korhonen, A., … Elenius, K. (2007). Association of Wwox with ErbB4 in Breast Cancer. *Cancer Research*, *67*(19), 9330–9337.

Arnaout, A. H., Dawson, P. M., Soomro, S., Taylor, P., Theodorou, N. A., Feldmann, M., Shousha, S. (1992). HER2 (c-erbB-2) oncoprotein expression in colorectal adenocarcinoma: an immunohistological study using three different antibodies. *Journal of Clinical Pathology*, *45*(8), 726–7.

Arnold, M., Sierra, M. S., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., & Thomas, A. (2017). Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, (66), 683–691.

Aufderheide, A. C. (2003). History of mummy studies. *The Scientific Study of Mummies*, 1–21.

Aykan, N. F. (2015). Red meat and colorectal cancer. *Oncology Reviews*, *9*(1), 38–44.

Azizah, A. M., Nor Saleha, I. T., Noor Hashimah, A., Asmah, Z. A., & Mastulu, W. (2016). Malaysian National Cancer Registry Report 2007-2011, Malaysia Cancer Statistics, Data and Figure. *National Cancer Institue*, 203.

Baggaley, A. (Ed.). (2001). *Human Body: Anatomy of the human body (Digestive system)*. *A Dorling Kindersley Book* (1st Editio). London: Dorling Kindersley Limited.

Balducci, L., & Ershler, W. B. (2005). Cancer and ageing: a nexus at several levels. *Nature Reviews. Cancer*, *5*(8), 655–662.

Bandrés, E., Zárate, R., Ramirez, N., Abajo, A., Bitarte, N., & García-Forcillas, J. (2007). Pharmacogenomics in colorectal cancer: The first step for individualized-therapy. *World Journal of Gastroenterology*, *13*(44), 5888–5901.

Bardhan, K., & Liu, K. (2013). Epigenetics and colorectal cancer pathogenesis. *Cancers*, *5*(2), 676–713.

Baretton, G. B., Diebold, J., Christoforis, G., Vogt, M., Dopfer, K., Schneiderbanger, K., Lohrs, U. (1996). Apoptosis and lmmunohistochemical bcl-2 Expression in Colorectal Adenomas and Carcinomas: Aspects of Carcinogenesis and Prognostic Significance. *Cancer*, *77*(2), 255–264.

Baselga, J., & Swain, S. M. (2009). Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nature Reviews Cancer*, *9*(7), 463–475.

Begnami, M. D., Fukuda, E., Fregnani, J. H. T. G., Nonogaki, S., Montagnini, A. L., da Costa, W. L., & Soares, F. A. (2011). Prognostic Implications of Altered Human Epidermal Growth Factor Receptors (HERs) in Gastric Carcinomas: HER2 and HER3 Are Predictors of Poor Outcome. *Journal of Clinical Oncology*, *29*(22), 3030–3036.

Beji, A., Horst, D., Engel, J., Kirchner, T., & Ullrich, A. (2012a). Toward the prognostic significance and therapeutic potential of HER3 receptor tyrosine kinase in human colon cancer. *Clinical Cancer Research*, *18*(4), 956–968.

Beji, A., Horst, D., Engel, J., Kirchner, T., & Ullrich, A. (2012b). Toward the prognostic significance and therapeutic potential of HER3 receptor tyrosine kinase in human colon cancer. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *18*(4), 956–68.

Bellacosa, A., Kumar, C. C., Di Cristofano, A., & Testa, J. R. (2005). Activation of AKT kinases in cancer: implications for therapeutic targeting. *Adv Cancer Res*, *94*, 29–86. http://doi.org/S0065-230X(05)94002-5 [pii]\r10.1016/S0065-230X(05)94002-5

Bennasroune, A., Gardin, A., Aunis, D., Crémel, G., & Hubert, P. (2004). Tyrosine kinase receptors as attractive targets of cancer therapy. *Critical Reviews in Oncology/hematology*, *50*(1), 23–38.

Berney, C. R., Fisher, R. J., Yang, J., Russell, P. J., & Crowe, P. J. (1999). Protein Markers in Colorectal Cancer Predictors of Liver Metastasis. *Annals of Surgery*, *230*(2), 179–184.

Blok, E. J., Kuppen, P. J. K., van Leeuwen, J. E. M., & Sier, C. F. M. (2013). Cytoplasmic overexpression of HER2: A key factor in colorectal cancer. *Clinical Medicine Insights: Oncology*, *7*, 41–51.

Brevet, M., Arcila, M., & Ladanyi, M. (2010). Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. *J Mol Diagn*, *12*(2), 169–176.

Bukholm, I. K., & Nesland, J. M. (2000). Protein expression of p53, p21 (WAF1/CIP1), bcl-2, Bax, cyclin D1 and pRb in human colon carcinomas. *Virchows Archiv*, *436*(3), 224–228.

Bussu, F., Ranelletti, F. O., Gessi, M., Graziani, C., Lanza, P., Lauriola, L., Almadori, G. (2012). Immunohistochemical expression patterns of the HER4 receptors in normal mucosa and in laryngeal squamous cell carcinomas: Antioncogenic significance of the HER4 protein in laryngeal squamous cell carcinoma. *Laryngoscope*, *122*(8), 1724–1733.

Caley, A., & Jones, R. (2012). The principles of cancer treatment by chemotherapy. *Surgery (Oxford)*, *30*(4), 186–190.

Cameron, E. A. (2011). Endoscopy short topics. *Medicine*, *39*(5), 293–295.

Campbell, M. R., Amin, D., & Moasser, M. M. (2010). HER3 comes of age: new insights into its functions and role in signaling, tumor biology, and cancer therapy. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *16*(5), 1373–83.

Cang, S., Iragavarapu, C., Savooji, J., Song, Y., & Liu, D. (2015). ABT-199 (venetoclax) and BCL-2 inhibitors in clinical development. *Journal of Hematology & Oncology*, *8*(1), 129.

Cappuzzo, F., Toschi, L., Domenichini, I., Bartolini, S., Ceresoli, G. L., Rossi, E., Varella-Garcia, M. (2005). HER3 genomic gain and sensitivity to gefitinib in advanced non-small-cell lung cancer patients. *British Journal of Cancer*, *93*(12), 1334–40. http://doi.org/10.1038/sj.bjc.6602865

Cho, E., Lee, J. E., Rimm, E. B., Fuchs, C. S., & Giovannucci, E. L. (2012). Alcohol consumption and the risk of colon cancer by family history of colorectal cancer. *American Journal of Clinical Nutrition*, *95*(2), 413–419.

Ciardiello, F., & Tortora, G. (2008). EGFR Antagonists in Cancer Treatment. *New England Journal of Medicine*, *358*(11), 1160–1174.

Compton, C. C. (2003). Colorectal carcinoma: diagnostic, prognostic, and molecular features. *Modern Pathology*, *16*(4), 376–388.

Compton, C. C., & Greene, F. L. (2004). The Staging of Colorectal Cancer: 2004 and Beyond. *CA: A Cancer Journal for Clinicians*, *54*(6), 295–308.

Culp, M. (2000). Antigen-Antibody Interaction: Application of the Ouchterlony Method for Determining Evolutionary Relationships. *The American Biology Teacher*, *62*(9), 658–663.

Cunningham, C., & Lindsey, I. (2007). Colorectal cancer : management. *Medicine*, *35*(6), 306–310.

David, A. R., & Zimmerman, M. R. (2010). Cancer: an old disease, a new disease or something in between? *Nature Reviews. Cancer*, *10*(10), 728–733.

Demirbaş, S., Sücüllü, I., Yildirim, S., & Celenk, T. (2006). Influence of the c-erb B-2, nm23, bcl-2 and p53 protein markers on colorectal cancer. *The Turkish Journal of Gastroenterology*, *17*(1), 13–9.

Derry, M. M., Raina, K., Agarwal, C., & Agarwal, R. (2013). Identifying molecular targets of lifestyle modifications in colon cancer prevention. *Frontiers in Oncology*, *3*(May), 119.

Desbois-Mouthon, C. (2010). The HER3/ErbB3 receptor: a promising target in cancer drug therapy. *Gastroenterologie Clinique et Biologique*, *34*(4–5), 255–259.

Drecoll, E., Nitsche, U., Bauer, K., Berezowska, S., Slotta-Huspenina, J., Rosenberg, R., & Langer, R. (2014). Expression analysis of heat shock protein 90 (HSP90) and Her2 in colon carcinoma. *International Journal of Colorectal Disease*, *29*(6), 663–71.

Dunstan, R. W., Wharton, K. A., Quigley, C., & Lowe, A. (2011). The Use of Immunohistochemistry for Biomarker Assessment—Can It Compete with Other Technologies? *Toxicologic Pathology*, *39*(6), 988–1002.

Duraiyan, J., Govindarajan, R., Kaliyappan, K., & Palanisamy, M. (2012). Applications of immunohistochemistry. *Journal of Pharmacy and Bioallied Sciences*, *4*(6), 307.

Edwards, C. L., Fillingim, R. B., & Keefe, F. (2001). Race, ethnicity and pain. *Pain*, *94*(2), 133–137.

Ellina, M.-I., Bouris, P., Aletras, A. J., Theocharis, A. D., Kletsas, D., & Karamanos, N. K. (2014). EGFR and HER2 exert distinct roles on colon cancer cell functional properties and expression of matrix macromolecules. *Biochimica et Biophysica Acta*, *1840*(8), 2651–61.

Ellis, H., & Mahadevan, V. (2014). Anatomy of the caecum, appendix and colon. *Surgery (Oxford)*, *32*(4), 155–158.

Field, A. E., Coakley, E. H., Must, A., Spadano, J. L., Laird, N., Deitz, W., Colditz, G. (2001). Impact of Overweight on the Risk of Developing Common Chronic Diseases During a 10-Year Period. *Archives of Internal Medicin*, *161*(3), 1581–1586.

Gala, K., & Chandarlapaty, S. (2014). Molecular pathways: HER3 Targeted therapy. *Clinical Cancer Research*, *20*(6), 1410–1416.

Garza-Treviño, E. N., Said-Fernández, S. L., & Martínez-Rodríguez, H. G. (2015). Understanding the colon cancer stem cells and perspectives on treatment. *Cancer Cell International*, *15*(1), 2.

Gespach, C. (2011). Increasing potential of HER3 signaling in colon cancer progression and therapy. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *18*(4), 917–919.

Ghahremani, G. G., White, E. M., Hoff, F. L., Gore, R. M., Miller, J. W., & Christ, M. L. (1992). Appendices epiploicae of the colon: radiologic and pathologic features. *Radiographics : A Review Publication of the Radiological Society of North America, Inc*, *12*(1), 59–77.

Gilmour, L. M. R., Macleod, K. G., Mccaig, A., Gullick, W. J., Smyth, J. F., & Langdon, S. P. (2001). Expression of erbB-4 / HER-4 Growth Factor Receptor Isoforms in Ovarian Cancer, *1264*, 2169–2176.

Giovannucci, E., & Michaud, D. (2007). The Role of Obesity and Related Metabolic Disturbances in Cancers of the Colon, Prostate, and Pancreas. *Gastroenterology*, *132*(6), 2208–2225.

Giovannucci, E., Rimm, E. B., Ascherio, A., Stampfer, M. J., Colditz, G. A., & Willett, W. C. (1995). Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *Journal of the National Cancer Institute*, *87*(4), 265–273.

Guo, G. F., Cai, Y. C., Zhang, B., Xu, R. H., Qiu, H. J., Xia, L. P., Wang, F. (2011). Overexpression of SGLT1 and EGFR in colorectal cancer showing a correlation with the prognosis. *Medical Oncology*, *28*(S1), 197–203.

Half, E., Broaddus, R., Danenberg, K. D., Danenberg, P. V., Ayers, G. D., & Sinicrope, F. A. (2004). HER-2 receptor expression, localization, and activation in colorectal cancer cell lines and human tumors. *International Journal of Cancer*, *108*(4), 540–548.

Hall, C., Troutman, S. M., Price, D. K., Figg, W. D., & Kang, M. H. (2013). Bcl-2 Family of Proteins as Therapeutic Targets in Genitourinary Neoplasms. *Clinical Genitourinary Cancer*, *11*(1), 10–19.

Hall, N. (2011). Colorectal cancer: Features and investigation. *Medicine*, *39*(5), 250–253.

Hamilton, S. R., & Aaltonen, L. A. (Eds.). (2000). *World Health Organization Classification of Tumours Pathology and Genetics of Tumours of the Digestive System*. IARC Press: Lyon 2000.

Han, H. S., Park, Y. M., & Hwang, T. S. (2006). Differential expression of Bcl-2, Bcl-XL and p53 in colorectal cancer. *Journal of Gastroenterology and Hepatology (Australia)*, *21*(7), 1108–1114.

Hanahan, D., & Weinberg, R. a. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646–74.

Haq, A. I., Schneeweiss, J., Kalsi, V., & Arya, M. (2009). The Dukes staging system: a cornerstone in the clinical management of colorectal cancer. *The Lancet. Oncology*, *10*(11), 1128.

Hayashi, M., Inokuchi, M., Takagi, Y., Yamada, H., Kojima, K., Kumagai, J., Sugihara, K. (2008). High expression of HER3 is associated with a decreased survival in gastric cancer. *Clinical Cancer Research*, *14*(23), 7843–7849.

Hecht, S. S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Reviews. Cancer*, *3*(10), 733–44.

Hector, S., & Prehn, J. H. M. (2009). Apoptosis signaling proteins as prognostic biomarkers in colorectal cancer: a review. *Biochimica et Biophysica Acta*, *1795*(2), 117–29.

Herreros-Villanueva, M., Rodrigo, M., Claver, M., Muñiz, P., Lastra, E., García-Girón, C., & Coma Del Corral, M. J. (2011). KRAS, BRAF, EGFR and HER2 gene status in a Spanish population of colorectal cancer. *Molecular Biology Reports*, *38*(2), 1315–1320.

Hirsch, F. R., Varella-Garcia, M., Bunn, P. A., Di Maria, M. V., Veve, R., Bremnes, R. M., Franklin, W. A. (2003). Epidermal Growth Factor Receptor in Non–Small-Cell Lung Carcinomas: Correlation Between Gene Copy Number and Protein Expression and Impact on Prognosis. *Journal of Clinical Oncology*, *21*(20), 3798–3807.

Hoeijmakers, J. H. J. (2009). DNA damage, aging, and cancer. *New England Journal of Medicine*, *361*(15), 1475–1485.

Hollmén, M., & Elenius, K. (2010). Potential of ErbB4 antibodies for cancer therapy. *Future Oncology (London, England)*, *6*(1), 37–53.

Horton, J. K., & Tepper, J. E. (2005). Staging of Colorectal Cancer: Past, Present, and Future. *Clinical Colorectal Cancer*, *4*(5), 302–312.

Huang, S., Li, C., Armstrong, E. a, Peet, C. R., Saker, J., Amler, L. C., … Harari, P. M. (2013). Dual targeting of EGFR and HER3 with MEHD7945A overcomes acquired resistance to EGFR inhibitors and radiation. *Cancer Research*, *73*(2), 824–33.

Hung, M., & Link, W. (2011). Protein localization in disease and therapy. *Journal of Cell Science*, *124*(20), 3381–3392.

Iqbal, N., & Iqbal, N. (2014). Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. *Molecular Biology International*, *2014*, 1–9.

Ishibashi, H., Suzuki, T., Suzuki, S., Moriya, T., Kaneko, C., Takizawa, T., Sasano, H. (2003). Sex Steroid Hormone Receptors in Human Thymoma. *The Journal of Clinical Endocrinology & Metabolism*, *88*(5), 2309–2317.

Jiang, D., Li, X., Wang, H., Shi, Y., Xu, C., Lu, S., … Tan, L. (2015). The prognostic value of EGFR overexpression and amplification in Esophageal squamous cell Carcinoma. *BMC Cancer*, *15*(1), 377.

Kapitanovic, S., Radosevic, S., Kapitanovic, M., Andelinovic, S., Ferencic, Z., Tavassoli, M., Spaventi, R. (1997). The expression of p185(HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. *Gastroenterology*, *112*(4), 1103–1113.

Kapitanović, S., Radošević, S., Slade, N., Kapitanović, M., Anđelinović, Š., Ferenčić, Ž., Spaventi, R. (2000). Expression of erbB-3 protein in colorectal adenocarcinoma: correlation with poor survival. *Journal of Cancer Research and Clinical Oncology*, *126*(4), 205–211.

Kavanagh, D. O., Chambers, G., O'Grady, L., Barry, K. M., Waldron, R. P., Bennani, F., Tobbia, I. (2009). Is overexpression of HER-2 a predictor of prognosis in colorectal cancer? *BMC Cancer*, *9*, 1.

Kavuri, S. M., Jain, N., Galimi, F., Cottino, F., Leto, S. M., Migliardi, G., Bose, R. (2015). HER2 activating mutations are targets for colorectal cancer treatment. *Cancer Discovery*, *5*(8), 832–841.

Kay, E. W., Mulcahy, H., Walsh, C. B., Leader, M., & O'donoghue, D. (1994). Cytoplasmic c-erbB-2 protein expression correlates with survival in Dukes' B colorectal carcinoma. *Histopathology*, *25*(5), 455–461.

Kim, H.-P., Yoon, Y.-K., Kim, J.-W., Han, S.-W., Hur, H.-S., Park, J., Kim, T.-Y. (2009). Lapatinib, a Dual EGFR and HER2 Tyrosine Kinase Inhibitor, Downregulates Thymidylate Synthase by Inhibiting the Nuclear Translocation of EGFR and HER2. *PLoS ONE*, *4*(6), e5933.

Koehler, B. C., Scherr, A.-L., Lorenz, S., Urbanik, T., Kautz, N., Elssner, C., Schulze-Bergkamen, H. (2013). Beyond cell death - antiapoptotic Bcl-2 proteins regulate migration and invasion of colorectal cancer cells in vitro. *PloS One*, *8*, e76446.

Kol, A., Terwisscha van Scheltinga, A. G. T., Timmer-Bosscha, H., Lamberts, L. E., Bensch, F., de Vries, E. G. E., & Schröder, C. P. (2014). HER3, serious partner in crime: therapeutic approaches and potential biomarkers for effect of HER3-targeting. *Pharmacology & Therapeutics*, *143*(1), 1–11.

Kountourakis, P., Pavlakis, K., Psyrri, A., Rontogianni, D., Xiros, N., Patsouris, E., Economopoulos, T. (2006). Clinicopathologic significance of EGFR and Her-2/neu in colorectal adenocarcinomas. *Cancer Journal*, *12*(3), 229–236.

Kountourakis, P., Pavlakis, K., Psyrri, A., Rontogianni, D., Xiros, N., Patsouris, E., Economopoulos, T. (2006). Prognostic significance of HER3 and HER4 protein expression in colorectal adenocarcinomas. *BMC Cancer*, *6*, 46.

Kouraklis, G., Kakisis, J., Theoharis, S., Tzonou, A., Glinavou, A., Raftopoulos, J., & Karatzas, G. (2003). Prognostic Significance and Correlation with Survival of bcl-2 and TGF-β RII in Colon cancer. *Digestive Diseases and Sciences*, *48*(12), 2284–2289.

Kruszewski, W. J., Rzepko, R., Ciesielski, M., Szefel, J., Zieliński, J., Szajewski, M., Wojtacki, J. (2010). Expression of HER2 in colorectal cancer does not correlate with prognosis. *Disease Markers*, *29*, 207–212.

Labianca, R., Beretta, G. D., Kildani, B., Milesi, L., Merlin, F., Mosconi, S., Wils, J. (2010). Colon cancer. *Critical Reviews in Oncology / Hematology*, *74*(2), 106–133.

Lam, L. T., Zhang, H., & Chyla, B. (2012). Biomarkers of therapeutic response to BCL2 antagonists in cancer. *Molecular Diagnosis and Therapy*, *16*(6), 347–356.

Lane, a, Segura-Cabrera, a, & Komurov, K. (2013). A comparative survey of functional footprints of EGFR pathway mutations in human cancers. *Oncogene*, (August), 1–12.

Larsson, S. C., Orsini, N., & Wolk, A. (2005). Diabetes mellitus and risk of colorectal cancer: A meta-analysis. *Journal of the National Cancer Institute*, *97*(22), 1679–1687.

Lédel, F., Hallström, M., Ragnhammar, P., Öhrling, K., & Edler, D. (2014). HER3 expression in patients with primary colorectal cancer and corresponding lymph node metastases related to clinical outcome. *European Journal of Cancer (Oxford, England : 1990)*, *50*(3), 656–62.

Lee, J. ., Wang, S.-T., Chow, N.-H., & Yang, H.-B. (2002). Investigation of the prognostic value of coexpressed erbB family members for the survival of colorectal cancer patients after curative surgery. *European Journal of Cancer*, *38*(8), 1065–1071.

Lee, M. Y., Lin, K. Der, Hsiao, P. J., & Shin, S. J. (2012). The association of diabetes mellitus with liver, colon, lung, and prostate cancer is independent of hypertension, hyperlipidemia, and gout in Taiwanese patients. *Metabolism: Clinical and Experimental*, *61*(2), 242–249.

Lee, W.-S., Park, Y. H., Lee, J. N., Baek, J., Lee, T., & Ha, S. Y. (2014). Comparison of HER2 expression between primary colorectal cancer and their corresponding metastases. *Cancer Medicine*, *3*(3), 674–680.

Li, A. R., Chitale, D., Riely, G. J., Pao, W., Miller, V. A., Zakowski, M. F., Samples, T. (2008). EGFR Mutations in Lung Adenocarcinomas Clinical Testing Experience and Relationship to EGFR Gene. *The Journal of Molecular Diagnostics*, *10*(3), 242–248.

Li, Q., Wang, D., Li, J., & Chen, P. (2011). Clinicopathological and prognostic significance of HER-2/neu and VEGF expression in colon carcinomas. *BMC Cancer*, *11*(1), 1–6.

Li, S., Buchbinder, E., Wu, L., Bjorge, J. D., Fujita, D. J., & Zhu, S. (2014). EGFR and HER2 levels are frequently elevated in colon cancer cells. *Discoveries Reports*, *1*(1), 1–8.

Liang, P. S., Chen, T., & Giovannucci, E. (2009). Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. *International Journal of Cancer. Journal International Du Cancer*, *124*(10), 2406–15.

Limsui, D., Vierkant, R. A., Tillmans, L. S., Wang, A. H., Weisenberger, D. J., Laird, P. W., Limburg, P. J. (2010). Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. *Journal of the National Cancer Institute*, *102*(14), 1012–1022.

Ljuslinder, I., Malmer, B., Isaksson-Mettävainio, M., Oberg, A., Henriksson, R., Stenling, R., & Palmqvist, R. (2009). ErbB 1-4 expression alterations in primary colorectal cancers and their corresponding metastases. *Anticancer Research*, *29*(5), 1489–94.

Matos, L. L. de, Trufelli, D. C., de Matos, M. G. L., & da Silva Pinhal, M. A. (2010). Immunohistochemistry as an important tool in biomarkers detection and clinical practice. *Biomarker Insights*, *5*, 9–20.

McKay, J. A., Murray, L. J., Curran, S., Ross, V. G., Clark, C., Murray, G. I., McLeod, H. L. (2002). Evaluation of the epidermal growth factor receptor (EGFR) in colorectal tumours and lymph node metastases. *European Journal of Cancer*, *38*(17), 2258–2264.

Memon, A. A., Sorensen, B. S., Melgard, P., Fokdal, L., Thykjaer, T., & Nexo, E. (2004). Expression of HER3, HER4 and their ligand heregulin-4 is associated with better survival in bladder cancer patients. *British Journal of Cancer*, *91*(12), 2034–2041.

Mescher, A. L. (2013). *Digestive tract: Large intestine*. *Junqueira's Basic Histology Text & Atlas* (13th Edition). McGraw Hill Lange: Medical.

Meterissian, S. H., Kontogiannea, M., Al-sowaidi, M., Linjawi, A., Halwani, F., Jamison, B., & Edwardes, M. (2001). Bcl-2 Is a Useful Prognostic Marker in Dukes ' B Colon Cancer. *Ann Surg Oncol*, *8*(6), 533–537.

Mill, C. P., Gettinger, K. L., & Riese, D. J. (2011). Ligand stimulation of ErbB4 and a constitutively-active ErbB4 mutant result in different biological responses in human pancreatic tumor cell lines. *Experimental Cell Research*, *317*(4), 392–404.

Naing, L., Winn, T., & Rusli, B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences, Medical Statistic*, *1*, 9–14.

Newton, K. F., Newman, W., & Hill, J. (2012). Review of biomarkers in colorectal cancer. *Colorectal Disease*, *14*(1), 3–17.

Normanno, N., De Luca, A., Bianco, C., Strizzi, L., Mancino, M., Maiello, M. R., Salomon, D. S. (2006). Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene*, *366*(1), 2–16.

Obrocea, F., Sajin, M., Marinescu, E., & Stoica, D. (2011). Colorectal cancer and the 7 th revision of the TNM staging system : review of changes and suggestions for uniform pathologic reporting. *Romanian Journal of Morphology & Embryology*, *52*(2), 537–544.

Ooi, A., Takehana, T., Li, X., Suzuki, S., Kunitomo, K., Iino, H., Dobashi, Y. (2004). Protein overexpression and gene amplification of HER-2 and EGFR in colorectal cancers: an immunohistochemical and fluorescent in situ hybridization study. *Modern Pathology : An Official Journal of the United States and Canadian Academy of Pathology, Inc*, *17*(8), 895–904.

Osako, T., Miyahara, M., Uchino, S., Inomata, M., Kitano, S., & Kobayashi, M. (1998). Immunohistochemical Study of c-erbB-2 Protein in Colorectal Cancer and the Corelation with Patient Survival. *Onocology*, *55*, 548–555.

Paskett, E. D., Reeves, K. W., Rohan, T. E., Allison, M. A., Williams, C. D., Messina, C. R., Hunt, J. R. (2007). Association between cigarette smoking and colorectal cancer in the Women's Health Initiative. *Journal of the National Cancer Institute*, *99*(22), 1729–1735.

Pawson, T. (1997). Signaling Through Scaffold, Anchoring, and Adaptor Proteins. *Science*, *278*(5346), 2075–2080.

Pericleous, M., Mandair, D., & Caplin, M. E. (2013). Diet and supplements and their impact on colorectal cancer. *Journal of Gastrointestinal Oncology*, *4*(4), 409–423.

Planas-Silva, M. D., Bruggeman, R. D., Grenko, R. T., & Smith, J. S. (2007). Overexpression of c-Myc and Bcl-2 during progression and distant metastasis of hormone-treated breast cancer. *Experimental and Molecular Pathology*, *82*(1), 85–90.

Poincloux, L., Durando, X., Seitz, J. F., Thivat, E., Bardou, V.-J., Giovannini, M.-H., Monges, G. (2009). Loss of Bcl-2 expression in colon cancer: a prognostic factor for recurrence in stage II colon cancer. *Surgical Oncology*, *18*(4), 357–65.

Pozzi, C., Cuomo, A., Spadoni, I., Magni, E., Silvola, A., Conte, A., Rescigno, M. (2016). The EGFR-specific antibody cetuximab combined with chemotherapy triggers immunogenic cell death. *Nature Medicine*, *22*(6), 624–631.

Rashid, M. R. a, Aziz, A. F. A., Ahmad, S., Shah, S. A., & Sagap, I. (2009). Colorectal cancer patients in a tertiary referral centre in Malaysia: a five year follow-up review. *Asian Pacific Journal of Cancer Prevention : APJCP*, *10*(6), 1163–6.

Rego, R. L., Foster, N. R., Smyrk, T. C., Le, M., O'Connell, M. J., Sargent, D. J., Sinicrope, F. a. (2010). Prognostic effect of activated EGFR expression in human colon carcinomas: comparison with EGFR status. *British Journal of Cancer*, *102*(1), 165–72.

Reimers, M. S., Zeestraten, E. C. M., Kuppen, P. J. K., Liefers, G. J., & van de Velde, C. J. H. (2013). Biomarkers in precision therapy in colorectal cancer. *Gastroenterology Report*, *1*(3), 166–83.

Roskoski, R. (2014). The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacological Research : The Official Journal of the Italian Pharmacological Society*, *79*, 34–74.

Sabounchi, S., Keihanian, S., & Anand, B. S. (2012). Impact of race on colorectal cancer. *Clinical Colorectal Cancer*, *11*(1), 66–70.

Saladin, K. S. (2016). *Human anatomy* (5th Edition). New York: Mc Graw Hill Education.

Schaefer, G., Haber, L., Crocker, L. M., Shia, S., Shao, L., Dowbenko, D., Eigenbrot, C. (2011). A Two-in-One Antibody against HER3 and EGFR Has Superior Inhibitory Activity Compared with Monospecific Antibodies. *Cancer Cell*, *20*(4), 472–486.

Schuell, B., Gruenberger, T., Scheithauer, W., Zielinski, C., & Wrba, F. (2006). HER 2/neu protein expression in colorectal cancer. *BMC Cancer*, *6*(1), 123.

Seo, A. N., Kwak, Y., Kim, D. W., Kang, S. B., Choe, G., Kim, W. H., & Lee, H. S. (2014). HER2 status in colorectal cancer: Its clinical significance and the relationship between HER2 gene amplification and expression. *PLoS ONE*, *9*.

Seo, A. N., Kwak, Y., Kim, W. H., Kim, D. W., Kang, S. B., Choe, G., & Lee, H. S. (2015). HER3 protein expression in relation to HER2 positivity in patients with primary colorectal cancer: Clinical relevance and prognostic value. *Virchows Archiv*, *466*(6), 645–654.

Sequist, L. V, & Lynch, T. J. (2008). EGFR tyrosine kinase inhibitors in lung cancer: an evolving story. *Annual Review of Medicine*, *59*, 429–442.

Seshacharyulu, P., Ponnusamy, M. P., Haridas, D., Jain, M., Ganti, A. K., & Batra, S. K. (2012). Targeting the EGFR signaling pathway in cancer therapy. *Expert Opinion on Therapeutic Targets*, *16*(1), 15–31.

Shaw, A. S., & Filbert, E. L. (2009). Scaffold proteins and immune-cell signalling. *Nature Reviews Immunology*, *9*(1), 47–56.

Sheng, Q., & Liu, J. (2011). The therapeutic potential of targeting the EGFR family in epithelial ovarian cancer. *British Journal of Cancer*, *104*(8), 1241–1245.

Shier, D., Butler, J., & Lewis, R. (2017). *Hole's Essentials of Human Anatomy & Physiology* (13th ed.). New York: Mc Graw Hill Education.

Sinicrope, F. A., Hart, J., Michelassi, F., & Lee, J. J. (1995). Prognostic value of bcl-2 oncoprotein expression in stage II colon carcinoma. *Clinical Cancer Research*, *1*(10), 1103–1110.

Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Norton, L. (2001). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *New England Journal of Medicine*, *344*(11), 783–792.

Spano, J. P. J.-P., Lagorce, C., Atlan, D., Milano, G., Domont, J., Benamouzig, R., Wind, P. (2005). Impact of EGFR expression on colorectal cancer patient prognosis and survival. *Annals of Oncology*, *16*(1), 102–108.

Srinivasan, R., Gillett, C. E., Barnes, D. M., & Gullick, W. J. (2000). Advances in Brief Nuclear Expression of the c-erbB-4 / HER-4 Growth Factor Receptor in Invasive Breast Cancers, (5), 1483–1487.

Srinivasan, R., Poulsom, R., Hurst, H. C., & Gullick, W. J. (1998). Expression of the c-erbB-4/HER4 protein and mRNA in normal human fetal and adult tissues and in a survey of nine solid tumour types. *Journal of Pathology*, *185*(October 1997), 236–245.

Srivastava, S., Verma, M., & Henson, D. E. (2001). Biomarkers for Early Detection of Colon Cancer Biomarkers for Early Detection of Colon Cancer. *Clinical Cancer Reserach*, *7*, 1118–1126.

Stewart, B. W. (1994). Mechanisms of apoptosis: integration of genetic, biochemical, and cellular indicators. *Journal of the National Cancer Institute*, *86*(17), 1286–96.

Su, T. T., Goh, J. Y., Tan, J., Muhaimah, A. R., Pigeneswaren, Y., Khairun, N. S., Majid, H. A. (2013). Level of colorectal cancer awareness: a cross sectional exploratory study among multi-ethnic rural population in Malaysia. *BMC Cancer*, *13*, 376.

Sudhakar, A. (2009). History of Cancer, Ancient and Modern Treatment Methods. *Journal of Cancer Science & Therapy*, *1*(2), 1–4.

Sun, N., Meng, Q., & Tian, A. (2010). Expressions of the anti-apoptotic genes Bag-1 and Bcl-2 in colon cancer and their relationship. *American Journal of Surgery*, *200*(3), 341–5.

Sung, J. (2007). Colorectal cancer screening: its time for action in Asia. *Cancer Detection and Prevention*, *31*(1), 1–2.

Sung, J. J., Lau, J. Y., Goh, K., & Leung, W. (2005). Increasing incidence of colorectal cancer in Asia: implications for screening. *The Lancet. Oncology*, *6*(11), 871–6.

Sung, J. J. Y., Ng, S. C., Chan, F. K. L., Chiu, H. M., Kim, H. S., Matsuda, T., … Goh, K. L. (2015). An updated Asia Pacific Consensus Recommendations on colorectal cancer screening. *Gut*, *64*(1), 121–132.

Tovey, S. M., Dunne, B., Witton, C. J., Cooke, T. G., & Bartlett, J. M. S. (2006). HER4 in breast cancer: comparison of antibodies against intra- and extra-cellular domains of HER4. *Breast Cancer Research : BCR*, *8*(2), R19.

Ueno, H., Mochizuki, H., Hashiguchi, Y., Ishiguro, M., Kajiwara, Y., Sato, T., Talbot, I. C. (2008). Histological Grading of Colorectal Cancer. *Annals of Surgery*, *247*(5), 811–818.

Veettil, S. K., Lim, K. G., Chaiyakunapruk, N., Ching, S. M., & Abu Hassan, M. R. (2016). Colorectal cancer in Malaysia: Its burden and implications for a multiethnic country. *Asian Journal of Surgery*.

Watson, A. J. M. (2006). An overview of apoptosis and the prevention of colorectal cancer. *Critical Reviews in Oncology/hematology*, *57*(2), 107–21.

Weitz, J., Koch, M., Debus, J., Höhler, T., Galle, P. R., & Büchler, M. W. (2005). Colorectal cancer. *The Lancet*, *365*(9454), 153–65.

Williams, C. S., Bernard, J. K., Beckler, M. D., Almohazey, D., Washington, M. K., Smith, J. J., & Frey, M. R. (2015). ERBB4 is over-expressed in human colon cancer and enhances cellular transformation. *Carcinogenesis*, *36*(7), 710–718.

Witton, C. J., Reeves, J. R., Going, J. J., Cooke, T. G., & Bartlett, J. M. (2003). Expression of the HER1–4 family of receptor tyrosine kinases in breast cancer. *The Journal of Pathology*, *200*(3), 290–297.

Workman, P., & Kaye, S. B. (2002). Translating basic cancer research into new cancer therapeutics. *Trends Mol. Med.*, *8*(4), 0.

Wray, C. M., Ziogas, A., Hinojosa, M. W., Le, H., Stamos, M. J., & Zell, J. A. (2009). Tumor subsite location within the colon is prognostic for survival after colon cancer diagnosis. *Diseases of the Colon and Rectum*, *52*(8), 1359–1366.

Xu, H., Yu, Y., Marciniak, D., Rishi, A. K., Sarkar, F. H., Kucuk, O., & Majumdar, A. P. N. (2005). Epidermal growth factor receptor (EGFR)– related protein inhibits multiple members of the EGFR family in colon and breast cancer cells. *Molecular Cancer Therapeutics*, *4*(March), 435–442.

Yarden, Y. (2001). Biology of HER2 and its importance in breast cancer. *Oncology*, *61 Suppl 2*(suppl 2), 1–13.

Youle, R. J., & Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nature Reviews Molecular Cell Biology*, *9*(1), 47–59.

Yu, D., & Hung, M. C. (2000). Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. *Oncogene*, *19*(53), 6115–6121.

Yuhara, H., Steinmaus, C., Cohen, S. E., Corley, D. A., Tei, Y., & Buffler, P. A. (2011). Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? *The American Journal of Gastroenterology*, *106*(11), 1911–1921.