REVIEW ON PEPTIDE CONJUGATED NANOPARTICLES TARGETED TO COLORECTAL CANCER

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ABSTRACT

This article aims to provide an overview focused on peptide conjugation nanoparticles targeted at colorectal cancer as a reference for professional nuclear medicine technologists and researchers. It outlines the identification of colorectal cancer; current occurrences all over the world and in Malaysia; current treatments; nanoparticle as a drug delivery system and nanoparticles for targeted colorectal cancer therapy; peptides and peptide-nanoparticles conjugation; cancer imaging and types of imaging techniques; in vitro and in vivo studies of peptide nanoparticles conjugate against colorectal cancer. Colorectal cancer (CRC) involving the large intestine and rectum has become one of the most common types of cancer worldwide. This type of cancer treatment still leads to severe side effects and significant death despite advances in medicine. The benefits of nanotechnology and the combined drug delivery style are still constrained without the active ligands required for treatment effectiveness. This paper also comments on the role of peptide ligands in the imaging and treatment of colorectal cancer targeting newly developed cells and tissues in the field of peptide-conjugated nanoparticles, discussing the promising use of peptide conjugated nanoparticles focused on CRC treatment.

ABSTRAK

Artikel ini bertujuan untuk memberikan gambaran keseluruhan yang difokuskan pada nanopartikel konjugasi peptida yang disasarkan pada barah kolorektal sebagai rujukan bagi ahli teknologi dan penyelidik perubatan nuklear profesional. Ia menggariskan pengenalan barah kolorektal; kejadian semasa di seluruh dunia dan di Malaysia; rawatan semasa; nanopartikel sebagai sistem penyampaian ubat dan nanopartikel untuk terapi barah kolorektal yang disasarkan; peptida dan konjugasi peptida-nanopartikel; pengimejan kanser dan jenis teknik pencitraan; kajian in vitro dan in vivo nanopartikel peptida konjugat terhadap barah kolorektal. Kanser kolorektal (CRC) yang melibatkan usus besar dan rektum telah menjadi salah satu jenis barah yang paling biasa di seluruh dunia. Rawatan barah jenis ini masih membawa kepada kesan sampingan yang teruk dan kematian yang ketara walaupun telah maju dalam bidang perubatan. Faedah nanoteknologi dan gabungan gaya penyampaian ubat masih dikekang tanpa ligan aktif yang diperlukan untuk keberkesanan rawatan. Makalah ini juga mengulas mengenai peranan ligan peptida dalam pencitraan dan rawatan barah kolorektal yang menargetkan sel dan tisu yang baru dikembangkan dalam bidang nanopartikel konjugasi peptida, membincangkan penggunaan menjanjikan nanopartikel konjugat peptida yang difokuskan pada rawatan CRC.

Keywords: Peptide-conjugated nanoparticles, colorectal cancer, drug delivery, targeted nanoparticles

INTRODUCTION

The colon is also known as the large bowel or large intestine is the distal part of the gastrointestinal tract, extending from the cecum to the anal canal. It is long, coiled, tube-like organ that is part of the digestive system in the human body. The digestive system is a group of organs that allow us to eat and to use the food we eat to fuel our body. It receives digested food from the small intestine, and its main function is to absorb water and electrolytes to form stool. The stool moves through the colon to the rectum and leave the body through the anus.

Anatomically, the colon can be divided into four parts namely ascending, transverse, descending and sigmoid [1]. These sections form an arch, which encircles the small intestine as in figure 1. The length of the average adult human colon is 65 inches or 166 cm (range of 80 to 313 cm) for males, and 61 inches or 155 cm (range of 80 to 214 cm) for females [2].

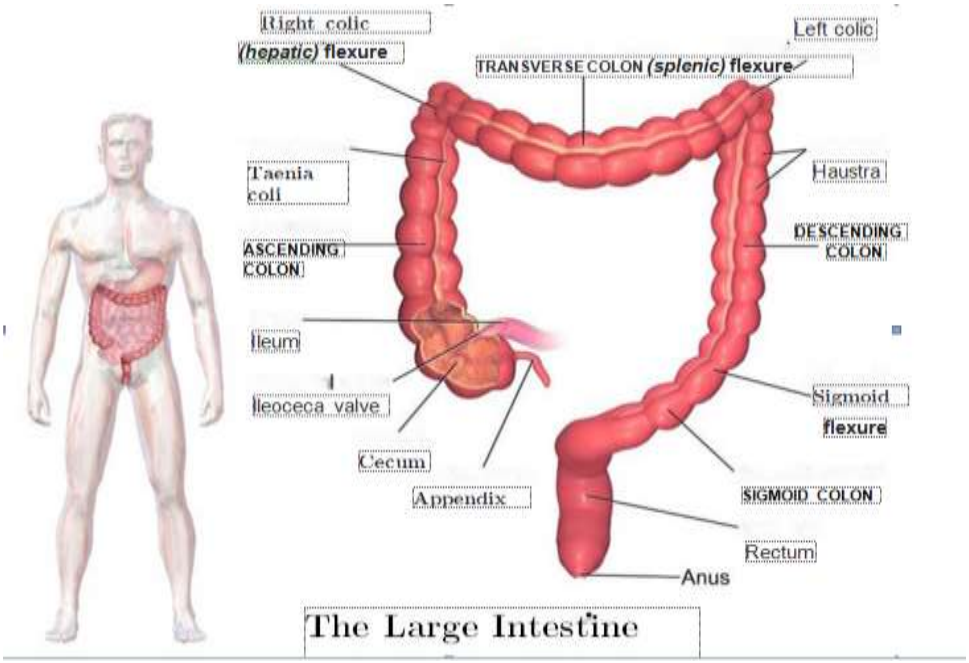

Figure 1. The large intestine contain 4 parts of colon [3]

The rectum is the concluding part of the large intestine that terminates at the anus. The average length of the human rectum may range approximately 10 to 15 cm. Its diameter can be compared to that of the sigmoid colon (the part of the large intestine located nearest to the rectum) at its onset. However, it becomes larger near the anus, where it forms the rectal ampulla [2].

A rectal examination may be conducted to diagnose certain diseases. Certain types of cancers may be diagnosed by performing an endoscopy in the rectum. An endoscopy is a procedure where a doctor uses an endoscope, a small, flexible tube with a camera and light to examine areas inside the body. Body temperature may also be obtained from the rectal area. In case of infants and babies, this is generally the most accurate method for determining actual body temperature.

Colorectal cancer (CRC), also known as bowel cancer, colon cancer, or rectal cancer, is the development of cancer from the colon or rectum. Colon cancer and rectal cancer are often grouped together because they have many features in common. Cancer is the abnormal growth of cells that have the ability to invade or spread to other parts of the body. Colorectal cancer may be benign, or non-cancerous, or malignant. A malignant cancer can spread to other parts of the body and damage them. The American Cancer Society estimates that about 1 in 21 men and 1 in 23 women in the United States will develop colorectal cancer during their lifetime. It is the second leading cause of cancer death in women, and the third for men [4].

The wall of the colon and rectum is made up of many layers. Most colorectal cancers start as a growth on the inner lining and can grow outward through some or all of the other layers of the colon or rectum. These growths are called polyps. Some types of polyps can change into cancer over time (usually many years), but not all polyps become cancer. The chances of a polyp changing into cancer depends on the type of polyp it is. There are 2 main types of polyps namely [5];

- Adenomatous polyps (adenomas): These polyps sometimes change into cancer. Because of this, adenomas are called a pre-cancerous condition.
- Hyperplastic polyps and inflammatory polyps: These polyps are more common, but in general they are not pre-cancerous.

Other factors that can make a polyp more likely to contain cancer or increase the risk of developing colorectal cancer include [5];

i. If a polyp is larger than 1 cm is found.
ii. If more than 2 polyps are found.
iii. If dysplasia is seen in the polyp after it is removed. Dysplasia is another pre-cancerous condition. It means there is an area in a polyp or in the lining of the colon or rectum where the cells look abnormal, but they do not look like true cancer cells.

When cancer cells are in the wall, they can then grow into blood vessels or lymph vessels. From there, they can travel to nearby lymph nodes or to distant parts of the body. The stages of a colorectal cancer depends on how deeply it grows into the wall and if it has spread outside the colon or rectum. Colorectal cancer is often a silent disease, developing with no symptoms at all. When symptoms do occur they may include the following indication [4];

- Blood in or on the stool
- Change in bowel habits
- Stools that is narrower than usual
- General stomach discomfort (bloating, fullness, and/or cramps)
- Vomiting
- Diarrhea, constipation, or feeling that the bowel does not empty completely
- Frequent gas pains
- Weight loss for no apparent reason
- Rectal bleeding
- Constant tiredness, or new fatigue during activity that was previously tolerated

Most colorectal cancers (about 95%) are considered sporadic, meaning the genetic changes develop by chance after a person is born, so there is no risk of passing these genetic mutations on to one's children. Inherited colorectal cancers are less common (about 5%) and occur when genetic mutations are passed down from one generation to the next [6].

Risk factors for colorectal cancer are as follows;

1. Age and gender - More than 90% of colorectal cancers occur in people older than 50. Men have a slightly higher risk of developing colorectal cancer than women.
2. Hereditary colorectal cancer syndrome - Members of families with certain uncommon inherited conditions also have a significantly increased risk of colorectal cancer, as well as other types of cancer. These include familial adenomatous polyposis, Gardner syndrome and Lynch syndrome.
3. Personal history of certain type of cancer - People with a personal history of colorectal cancer and women who have had ovarian cancer or uterine cancer are more likely to develop colorectal cancer

themselves.

4. Smoking - Recent studies have shown that smokers are more likely to die from colorectal cancer than nonsmokers.
5. Family history of colorectal cancer - Colorectal cancer may run in the family if first- degree relatives (parents, brothers, sisters, and children) or other family members (grandparents, aunts, uncles, nieces, nephews, grandchildren, and cousins) have had colorectal cancer. This is especially true when family members are diagnosed with colorectal cancer before age 50.
6. Inflammatory bowel disease (IBD) - People with IBD, such as ulcerative colitis or Crohns disease are at increased risk of getting colorectal cancer.
   Adenomatous polyps (adenomas) - People who have had adenomas have a greater risk of additional polyps and of colorectal cancer.
7. Physical inactivity and obesity.

There are various types of treatments available in hospital to treat colorectal cancer. First is surgery. Surgical resection is the removal of tumor, some surrounding healthy tissue and nearby lymph node. Some patients may undergo laparoscopic procedure to examine the abdomen and/or to perform colectomy (removal of all or part of colon). The incisions are smaller, and the recovery time is shorter than with standard colon surgery. Second type of treatment is radiation therapy. Radiation therapy may be used before surgery, called neo-adjuvant therapy, to shrink the tumor, and so that it is easier to remove. It may also be used after surgery to destroy any remaining cancer cells. Chemotherapy is often given at the same time as radiation therapy, called chemo-radiotherapy, to increase the effectiveness of the radiation therapy. It can also be given before surgery to avoid colostomy or to reduce the chances of the cancer to recur. Meanwhile, in chemotherapy treatment, it will destroy cancer cells, usually by stopping the cancer cells ability to grow and divide. It may be given after surgery to eliminate any remaining cancer cells [6].

Another type of treatment is targeted therapy. This treatment targets the cancer specific genes, protein, or the tissue environment that contribute to cancer growth and survival. It will block the growth and spread of cancer cells while limiting the damage to healthy cells. Anti-angiogenesis therapy is a type of targeted therapy. This treatment is focused on stopping angiogenesis, which is the process of making new blood vessels. This is mainly because a tumor needs the nutrients delivered by blood vessels to grow and spread. The goal of anti-angiogenesis therapy is to starve the tumor. Another type of targeted therapy is epidermal growth factor receptor (EGFR) inhibitor. It will stop or slow down the growth of colorectal cancer [6].

Nowdays, based on Pashtoon Murtaza Kasi et al. (2018), the percentage of patients diagnosed with rectal or colon cancer in different age categories over the years showed a rising trend for individuals aged < 50. Most of these tumors were distal (rectum, left-sided colon, and right-sided colon were 49.8%, 28.8%, and 21.4%, respectively). This was more so for patients < 50 diagnosed with rectal cancer, which showed a linear increase at a rate of 0.26% per year ($P < .001$) [7].

Multinational cohort study involving four Asian countries/regions, namely Taiwan, Korea, Japan, and Hong Kong reported, in Taiwan (1995-2014), incidence of young-onset CRC significantly increased in both men (colon cancer: 4.9-9.7 per 100,000; rectal cancer: 4.0-8.3 per 100,000) and women (colon cancer: 5.1-9.7 per 100,000; rectal cancer: 3.8-6.4 per 100,000). In Korea (1999-2014), incidence of young-onset CRC significantly increased in both men (colon cancer: 5.0-10.4 per 100,000; rectal cancer: 4.9-14.0 per 100,000) and women (colon cancer: 4.1-9.6 per 100,000; rectal cancer: 4.1-9.1 per 100,000). The most pronounced change was observed with male rectal cancer, increasing by 3.9% per year in Taiwan (Average Annual Percentage Change (AAPC) + 3.9, 95% confidence interval + 3.3 to +4.5, $P < 0.05$) and 6.0% per year in Korea (AAPC +6.0, 95% confidence interval + 4.5 to +7.6, $P < 0.05$). Only a significant increase in rectal cancer was noted in Japan (male rectal cancer: 7.2-10.1 per 100,000, female rectal cancer 4.7-6.7 per 100,000) and Hong Kong (male rectal cancer: 4.4-7.0 per 100,000) [8].

Vui Heng Chong et al. (2015), stated, CRC is the most common gastrointestinal malignancy and is a significant cause of mortality. Its incidence is generally increasing in Asia. In Brunei, the mean age of diagnosis was

59.3±14.6 years old, incidences being slightly higher amongst men (57.6%) and Malays (67.1%). The most common tumor type was adenocarcinoma (96.4%). Rectal cancers accounted for 35.2% (n=372/1,056) of all cancers of the large bowel, and more men were affected than women [9].

While in Malaysia, the ten most common cancers are breast, colorectal, lung, lymphoma, nasopharynx, leukemia, prostate, liver, cervix uteri and ovary. Table 1 showed the number of colorectal cancer cases occurred from 2012 to 2016 for all residents in Malaysia [7].

Table 1: Ten most common cancers, all residents in Malaysia, 2012-2016 [10].

ICD-10	Sites	No.	%
C50	Breast	21.925	19.0
C18-21	Colorectal	15.515	13.5
C33-34	Trachea, brjonchus and lung	11.256	9.8
C81-85.C96	Lymphoma	5.830	5.1
C11	Nasopharynx	4.597	4.0
C91-95	Leukaemia	4.273	3.7
C61	Prostate	4.189	3.6
C22	Liver	4.033	3.5
C53	Cervix uteri	3.981	3.5
C56	Ovary	3.575	3.1
	Others	36.064	31.3
	Total	115,238	100.0

A total of 15,515 cases of colorectal cancer were registered for the period of 2012-2016 compared with 13,693 cases in 2007-2011 report as shown in Table 2. According to gender, colorectal cancer incidence was more common in males with 56.1% occurrence compared to females with 43.9% occurrence.

Table 2: Colorectal cancer incidence by year in Malaysia [10].

All Residents	Male				Female			
	No.	CR	ASR	CumR	No.	CR	ASR	CumR
2007-2011	7,646	11.7	14.6	1.8	6,047	9.5	11.1	1.3
2012-2016	8,701	127	14.8	18	6,814	10.2	11.1	13
2012	1,590	11.6	13.5	17	1,269	9.5	10.3	1.2
2013	1.628	11.8	13.4	16	1,290	9.6	10.2	1.2
2014	1,788	129	145	18	1,291	9.6	10.2	1.2
2015	1,787	127	140	17	1,401	10.2	10.6	1.3
2016	1,908	136	150	19	1,563	11.4	11.9	1.4

Figure 2 showed that the Chinese have the highest rate of colorectal cancer in both gender, but showing a decreasing trend in the following years. This is followed by the Malays and Indians, but in contradiction with the Chinese, the trends are slightly increased. While in Figure 3 and 4, it is shown that the colorectal cancer incidence increased with age and peaked at the age of 70 and above for both gender.

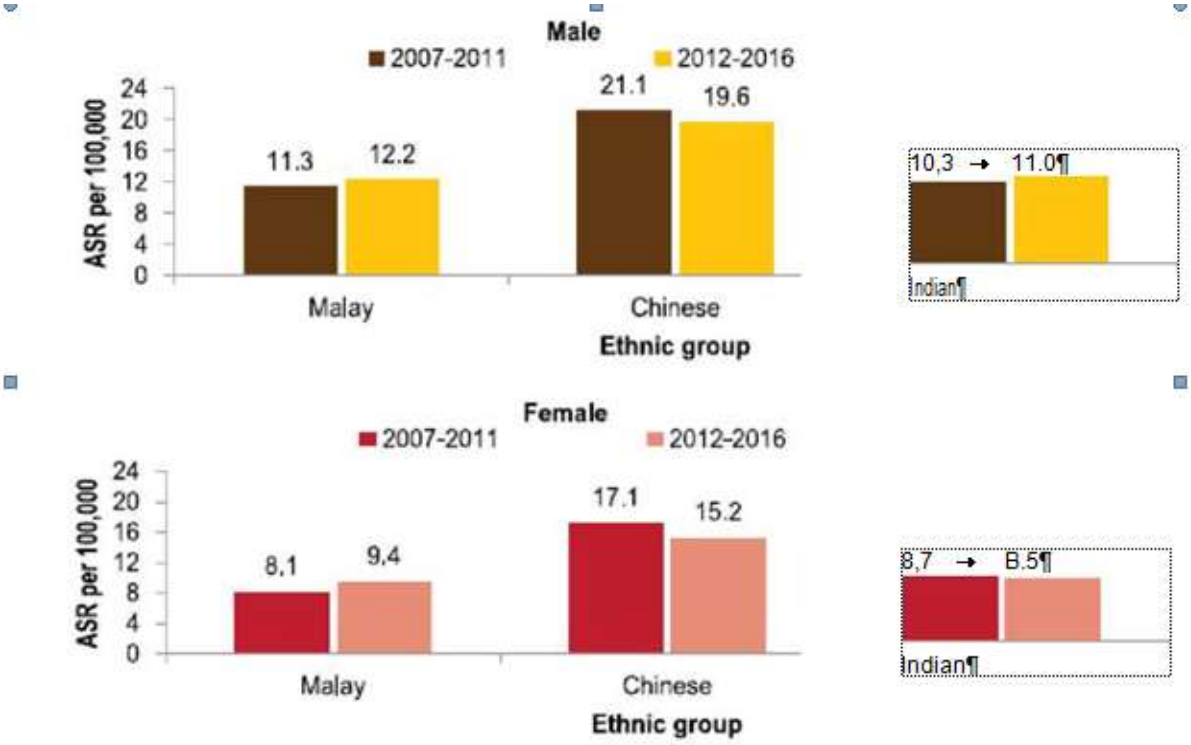

Figure 2: Colorectal cancer, comparison of age-standardized rate by year, major ethnic group and sex in Malaysia [10].

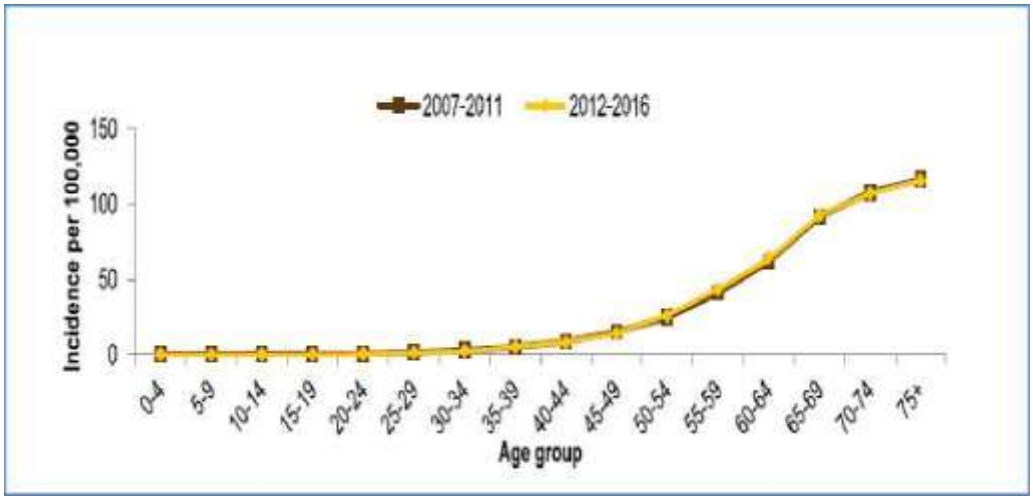

Figure 3: Colorectal cancer, comparison of age-specific incidence rate by year in male.

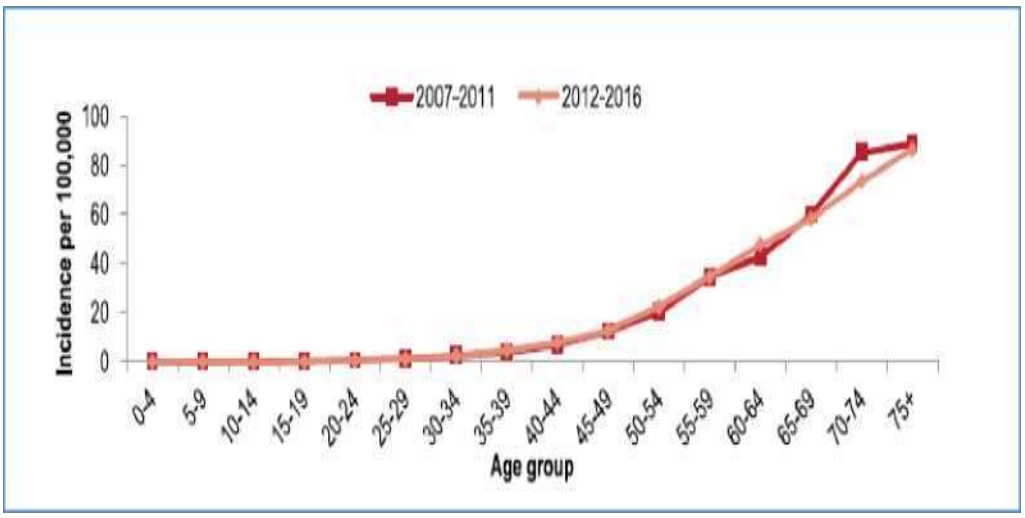

Figure 4: Colorectal cancer, comparison of age-specific incidence rate by year in female [10].

Cancer continues to be a leading cause of premature death and illness, which has a significant impact on the community, and health system. The cancer burden has increased due to several factors, such as population growth and ageing. It is also linked to economic and social development in a way of rapidly growing economies, particularly cancer related to poverty and lifestyle related infections caused-cancer mimic to industrialized countries. According to WHO, the need for urgent action to increase early-stage cancer detection, screening, and diagnosis to significantly improve cancer patients' chances of survival [10].

The trends of cancer incidence are probably a result of an array of factors such as lifestyle changes, population ageing, and a declining fertility rate. Lifestyle and behavioural factors such as obesity, physical inactivity, and smoking increase an individual's risk of developing cancer. Hence, it is important to encourage healthful behaviour in individuals to minimize the impact of these risk factors on health.

Consistently implementation and improving cancer awareness programs by emphasizing the importance of early diagnosis and encourage early help-seeking which are culturally appropriate can help the government or health sector to manage cancer prevention and treatment at an early stage.

CURRENT THERAPY FOR COLORECTAL CANCER

Treatment

Colorectal cancer is universally managed by a wide range treatment modality. Owing to the medical advancement that enables early detection and effective treatment, the mortality rate of colorectal cancer has declined progressively over the years. The treatment approach is essentially subjected to the biological manifestation of the cancerous cell, prognosis of the disease state, patient's performance status, accessibility, and affordability of the treatment options [11-14].

Surgery

Surgical resection has been the backbone of colorectal cancer management. The best curative outcome can be achieved by removal of the tumour [14, 15]. With the introduction of standardised surgery by complete excision of mesocolic with central vascular ligation in colorectal cancer, a reduction of 5-year local relapse rates from 6.5% to 3.6% and improvement of 5-year survival rates from 82.1 to 89.1% were accomplished [16]. Colectomy can be conducted via traditional open colorectal approach with large cut at abdomen or alternatively minimal invasive laparoscopic approach. Both methods have demonstrated insignificant difference in operative mortality, overall survival and disease-free survival. However, laparoscopic approach offers significant short-term benefits such as lower post-operative ileus rate, lesser intraoperative blood loss, shortened hospital stay and lower risk of post-operative wound infection [17, 18]. Novel robotic surgery may offer greater advantages, nevertheless, the significant greater cost has limit its application in selective patients [15, 18]. Despite the

highly recommended surgical approach, in circumstances where tumour is inoperable due to the unfavourable physiological conditions, size, site and metastasis of the tumour. Chemotherapy, radiotherapy or concurrent chemoradiotherapy may be alternative modalities before surgical approach is feasible [12-14].

Radiotherapy

Radiotherapy (RT) utilise high-energy and highly-precised radiation to destroy cancerous cells. This approach is not common in management of colon cancer but has more significant role in treating tumour originated from rectal. Both short and long course radiotherapy can be applied in rectal cancer. The 5-year Swedish Rectal Cancer Trial has reported 11% versus 27% of local recurrence rate and 74% versus 65% of overall survival in radiotherapy with surgery and surgery itself, respectively ($p<0.001$) and ($p=0.002$) [19]. Furthermore, a 10-year follow up study by Dutch Colorectal Cancer Group also concluded a significant fall in cumulative rate of local recurrence (5.6% vs 10.9%; $p=0.001$) and overall survival improvement (50% vs 40%; $p=0.032$) in patients underwent preoperative short course RT with surgery and surgery alone, respectively [20]. Neoadjuvant concurrent chemoradiation therapy (CCRT) was addressed in a large French randomised clinical trial on T3-4 rectal carcinoma. The supplementation of chemotherapy (Fluorouracil/Leucovorin) to radiotherapy (45 Gy) pre-operatively resulted in increased complete pathological response rate (11.4% vs 3.6%; $P<0.05$) and reduced 5-year incidence of local recurrence (8.1% vs 16.5%; $p<0.05$) compared with pre-operative RT alone [21].

Chemotherapy

Chemotherapy secures as the mainstay of pharmacological approach in colorectal cancer treatment. This vast group of treatment includes alkylating agents, antimetabolites, antimicrotubule agents, cytotoxic antibiotics and topoisomerase inhibitors. It functions by termination of cell division pathway through DNA separation and replication. Cytotoxic agents are generally non-specific drugs that destroy rapidly dividing cells in the body. Thus, chemotherapy is often associated with various unwanted adverse events [11]. 5-fluorouracil (5- FU) is opted as the most frequently administered cytotoxic compound in treatment of colorectal cancer. Adjuvant chemotherapy with 5-FU was shown to improve disease-free survival in Stage II (high risk) and Stage III patients [22]. Combination of oxaliplatin and 5-FU (FOLFOX) in the MOSAIC trial was discovered to be more superior than single 5-FU by improving six-year overall survive (72.9% vs 68.7%; $p=0.023$) and five-year disease-free survival (73.3% vs 67.4%; $p=0.003$) among Stage III colon cancer patients [23]. Addition of irinotecan to 5-FU (FOLFIRI) has also resulted in greater response rate (49% vs 31%; $p<0.001$) and median overall survival (17.4 vs 14.1 months; $p=0.031$) compared to 5-FU alone [24]. In certain cases where tumour reduction is required prior to surgical approach, a triplet antineoplastic combination of 5-FU, oxaliplatin and irinotecan (FOLFOXIRI) is used for a greater resection rate of the tumour [25].

Targeted therapy

Monoclonal antibodies target cancerous cell specifically with tolerable toxicity. This therapy inhibits the formation of blood vessel into tumour cells (angiogenesis) and disrupts the signalling pathways that mediate cell division. In general, monoclonal antibodies target on the epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF). Targeted therapy is typically utilised in combination with chemotherapy in the management of wildtype KRAS metastatic colorectal cancer [11]. The combination of bevacizumab with FOLFIRI has achieved an improvement in median overall survival (20.3 vs 15.6 months; $p<0.001$) and disease-free survival (10.6 vs 6.2 months; $p<0.001$) compared to FOLFIRI alone [26]. A multicenter, phase III FIRE-3 trial compared the combination of bevacizumab or cetuximab with FOLFIRI in patients with wildtype KRAS metastatic colorectal cancer. No statistically significant difference was concluded in median progression-free survival. However, the cetuximab arm established better overall survival than bevacizumab arm (28.7 vs 25.0 months; $p=0.017$) [27]. In recent years, the use of monoclonal antibodies as first or second line metastatic colorectal cancer treatment was not economically supported by the cost-effectiveness analysis [28, 29].

Immunotherapy

Treatment failure arises when patient develops therapeutic resistance towards conventional modalities. The progression of the cancer disease with 5-FU based chemotherapy and target therapy has accounted for an

approximately 6 months of median survival in patients. Hence, novel agent that target different pathways shall be explored in colorectal cancer [30]. The discovery of immunotherapy has greatly transformed the cancer treatment landscape. By secreting and recruiting immunosuppressive factors, tumour cells are safe from the destruction by immune surveillance. Pharmacologically, immunotherapy strengthens the capability of body immune system to detect, reject and destroy cancer cells with significant lesser treatment related side effects [31]. The current utilisation of immunotherapy is selective in tumour with elevated level of microsatellite instability (MSI-H) or deficient mismatch repair (dMMR). Programmed death-1 (PD-1) inhibitor is presently the most potential immune checkpoint inhibitor studied in colorectal cancer. Nivolumab, pembrolizumab and ipilimumab are also recommended in the National Comprehensive Network (NCCN) guidelines version 2.2019 for management of MSI-H or dMMR metastatic colorectal cancer that has advanced with chemotherapy [32, 33]. On-going phase M/MI clinical trials on immunotherapy in different disease setting and in combination with other treatment modalities will elucidate the role of immunotherapy in near future.

Nanoparticles as drug delivery system and nanoparticles for targeted colorectal cancer therapy

There is a need for an effective drug delivery system especially in cancer treatment. Nanoscale drug delivery such as nanoparticles and liposomes offer predictable, alleviate pharmacokinetic properties, sustained and maneuver release of drugs with low toxicity in systemic circulation. It has been found that liposome technology has potential benefit for patient with multidrug - resistant cancer.

Nanomedicine could enhance current limitations such as in diagnose, cancer treatment and management of critical diseases. Nanomedicines could benefit to an extend covering aspects such as tissues regeneration, drug delivery and imaging activities.

Liposomes is a closed spherical vesicle which consist of lipid bilayers which have the aqueous phase where the drug resides inside it. The diameter ranging from 400 nm to 2.5 uM whereas liposome diameter ranging from 1 to 100 nm. The objectives of nanoscale drug delivery system as in Table 3 [34].

It has been noted that high drug toxicity is a problem to the cancer treatment because the side effect resulting from the drug limit the dosage that can be administered to the patient as clearly shown by cytotoxic cancer drugs. Even the drug pass with flying colors in-vitro but it could be possibly act indiscriminately on both the cancerous and healthy tissues.

Table 3: Nanoscale drug delivery system objectives.

1)	To increase drug concentration at site of interest and minimize drug toxic level (if any) in healthy tissues.
2)	To improve the solubility of parenteral drug administration.
3)	To make sure there is constant rate of drug delivery at the site of action.
4)	Increasing drug half-life by reduce clearance.
5)	To heighten the drug stability (reduce degradation and maximize drug action).
6)	As vehicle for drug delivery across blood-brain barrier and blood-cochlear barrier.

Advantages of nanoscale drug delivery system and mechanism.

The ideal target for researcher is to find a nanoscale drug delivery system which ensure that the conjugated or bound drug-carrier complex reach the intended site and acts as planned. There is active targeting and passive targeting. In active targeting the complex incorporate specific ligand to the receptor or the epitope of interest whereas in passive targeting the complex diffuse and accumulate with excessive leaky microvasculature followed by subsequent extravasation of complex whereby the macromolecules are internalized from the blood point.

Biocompatibility and biodegradable play an important factor for any nanomaterials used in drug delivery. Renal excretion rapidly cleared the small particles (< 30 nm) whereas the mononuclear phagocytic system (MPS) cleared the bigger particles (> 30 nm). MPS consists of macrophages located in the liver and spleen which acts as phagocytotic scavengers. It also has been found that clearance also dependent on endothelial fenestral size. Fenestrae varies according to age, sex and genetics influence as it's link to their rate of clearance [34]. The surface properties of nanocarriers have significant effect on the rate of clearance by MPS thus Rutgers University have invented a method for evading opsonization of large nano carriers. The process is called PEGylation, a polymer, poly (ethylene glycol) (PEG; $[CH_2CH_2O]_n$ is conjugated to the drug carrier.

The drug therapeutic index (Eq. (1)) improved when there is ligand-drug-nanocarrier complexes. The high selectivity and specificity of the complexes resulting in the amount of the drug delivered to the target tissues increases and decrease in the amount of drug at the unwanted sites.

Drug therapeutic index = Maximum of non -toxic dose/ Minimum effective dose

NANOSCALE DRUG DELIVERY SYSTEM

Liposomes:

The bilayer could be composed of either synthetic or natural phospholipids. The interaction between water molecules and hydrophobic phosphate group of the phospholipids resulting in the lipid bilayer closes in on itself. According to [35], drug can be loaded into the liposomes by:

i) Saturated the liposomes formation in an aqueous solution in the presence of soluble drug.
ii) The use of proper organic solvents and solvents exchange mechanisms.
iii) Lipophilic drugs
iv) pH gradient method.

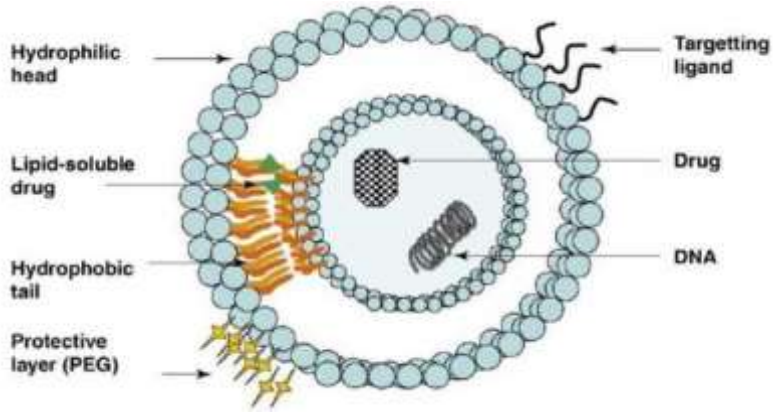

Figure 5: A bilaminar liposome. Drugs trapped in the hydrophobic region. The ligands of interest can be attached to the outer surface for active targeting or just PEGylated. The lipid bilayer numbers determine the liposomes categories, namely i) multimellar vesicles, ii) large unilamellar vesicles and iii) small unilamellar vesicles [34].

Both active and passive targeting strategies is viable for liposomes to reach target specific tissues. This is due to the liposomes are easily manipulated by the addition of additional molecules to the outer of lipid bilayer. Liposomes are in the range of 400 nm in size thus rapidly being cleared by the MPS system. If PEGylation was the choice, the opsonization will reduce thus reduces clearance by the MPS which lead to increasing the circulation half-life. Doxil® is an example for liposomal formulation for anticancer drugs which is to treat cancer in AIDS- related Kaposi sarcoma and multiple myeloma. Doxil® also under clinical trial for breast

cancer treatment. Recently Ogawara et al. [36] investigate effect of PEG liposomal doxorubicin and showed that PEG liposomal doxorubicin possesses anti-tumor effect on C26 colon cancer cells.

Another interesting development is the potential of liposomes to overcome multidrug resistance (MDR) acquired by cancers which in turn reduces chemotherapeutic efficacy. Increasing trend on resistance to chemotherapy render liposomes as effective treatment because researcher need not to find new chemotherapeutic drugs as the current drugs can be reformulate with the use of liposomes as nanocarrier. To date no specific citations regarding comparison study between liposomes and other nanoparticles delivery systems.

Solid lipid nanoparticles (SLNs):

Also known as lipospheres or solid lipid nanospheres which are solid lipid at human temperature 37°C with diameter between 50-1000 nm. Solid lipid nanoparticles can be formed from range of lipids including mono-, di- and triglycerides, fatty acids, waxes and any combination of the aforementioned.

SLNs are biodegradable and show biocompatibility and in addition low toxicity thus can be used in human. It has been found that SLNs need to be stabilized by surfactants in order to form administrable emulsions [37]. Factors that affect drug loading into SLN matrix are:

i) Solubility of the drug in lipid, must be lipophilic.

ii) Chemical and physical properties of the lipid or mixture of lipids.

iii) Depend on lipid crystalline characteristics at room temperature.

iv) Lipid(s) polymorphic form used.

Studies on delivery of various anticancer drugs have been done using SLNs and promising results using preclinical mouse trials have shown that SLNs might help in overcome MDR in cancers [38]. There is a study on benefits of SLNs in delivery of doxorubicin, cholesteryl butyrate and paclitaxel using colon cancer cells in vitro [39]. Also studies on mitoxantrone load on SLNs showed three folds reduction in lymph node metastases size compared to free mitoxantrone (not load into SLNs) [40].

SLNs is an alternative for drug delivery for cancers but more studies need to be done in order to safely translate its applications into human treatments.

Polymer-based NPs:

Polymer-based NPs is a class of NPs which is have been extensively investigated as drug nanocarriers. It's a polymeric backbone which usually formed from biodegradable monomers which is usually based on biocompatible simple organic molecule for active targeting, which is designed intercalated into the structures [41].

Drug load into polymeric NPs either by using i) polymer to form nanoscale structures by the entrapment of an aqueous drug phase [41, 42] or ii) chemical linking (simple esther or amide bond) between the drug molecules-polymer backbone that can be hydrolyzed in vivo. More complicated polymeric NPs involve polar groups for creating hydrophobic and hydrophilic region in order to enabling the drug to be absorbed and to facilitate delivery to the target site.

Polylactide (PLA) [43], poly(D,L-lactide-co-glycolide)(PLGA) [44] and PEG [45] are among the widely researched synthetic polymers. All the former polymers are hydrolyzed in vivo and biodegradable. There are also polymers that based on biological polysaccharides including chitosan, cyclodextrin and dextran [46]. Co-polymers can be formed combining different polymers. To facilitate active targeting, ligands are attached to NPs. By exploit cancer nature: specific antigens are expressed by cancer cells, the active targeting using ligands are involved. Increase drug delivery to prostate tumor cells was shown when RNA A10 aptamers specific antigens conjugated onto PLA-block-PEG co-polymers. There is potential for better non-surgical treatment for prostate cancer patients.

Current paclitaxel formulations Taxol® (breast cancer chemotherapy drug) show encapsulation efficacy of 70% when encapsulate with PEGylated PLGA copolymer NPs and showed similar level of apoptotic cell death when tested on HeLa cancer cells [47]. Previously Cremophor EL was used as organic solvent which elicit severe hypersensitivity reactions. In addition, PEGylated PLGA copolymer NPs showed no toxicity thus is much better than Cremophor EL without compromise the chemotherapeutic characteristic of Taxol®.

Another example is Cisplatin, an anticancer agent which has been loaded into copolymer PLGA-methoxy-PEG (PLGA-mPEG) NPs [48]. It has been found that cisplatin-loaded PLGA- mPEG NPs evoked less cytotoxicity than free cisplatin solution. In addition, cisplatin-loaded PLGA-mPEG NPs passively targeting LNCaP prostate cancer thus reduced systemic toxicity. Other studies using drug-carrying nanoparticles in this regard is PLGA (drug: Doxorubicin) [49], PLGA (drug: Dexamethasone) [50], PLA (drug: Thyrotropin releasing hormone) [51] and PLA- TPGS/MMT NP (drug: Docetaxel) [52].

Studies involving polymeric NPs still in preclinical phase, but it has potentials for targeted delivery of anticancer drug, relying to which ligands can be attached.

Gold NPs:

Gold nanoparticles consist a core of gold atom which can be functionalized by adding monolayer moieties comprise of thiol (SH) group [53]. Masked phosphonioalkyl selenoates [54], peptides and glyconanoparticles are among an example of these moieties which act as a ligand for active targeting of gold NPs.

Synthesize of gold NPs using $NaBH_4$ to reduce $AuCl_4^-$ salts in the presence of thiol containing moieties which later form a monolayer around the core atom as shown in Figure 6 [55]. Synthesized Gold NPs ranged 1-150 nm in diameter. Modification could be done by place exchange reaction by swapping thiol-containing moieties. This implies that single gold NPs core able to functionalized with many different groups for targeting, stability, evasion of host and drug delivery mechanism [55]. Connor, Mwamuka, Gole, Murphy and Wyatt (2005) had found that gold NPs are non-toxic at cellular level on numbers of human cell lines [56] whereas Hainfeld, Slatkin, Focella, and Smilowitz (2006) shown that in mice (as an imaging agent) no toxicity over 30 days [57].

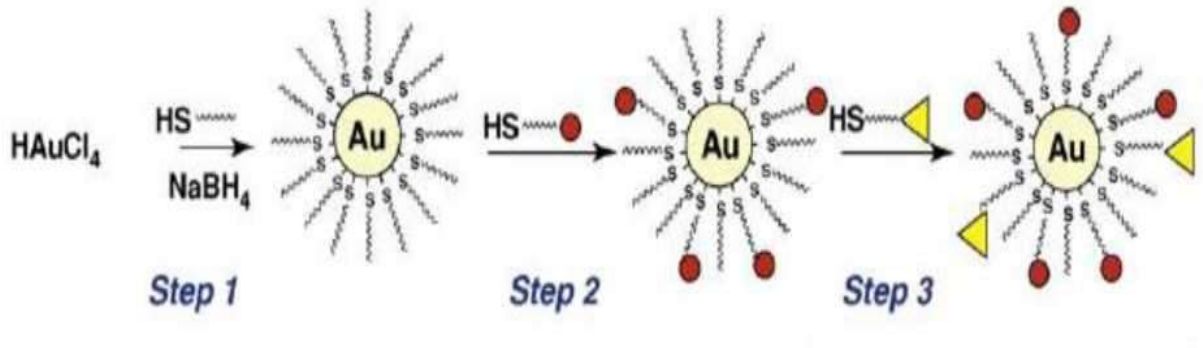

Figure 6: Synthesis of gold nanoparticles. Step 1 is Schiffrin reaction, $AuCl_4$" reduced by $NaBH_4$ in the presence of functional moieties, thiol group. Step 2 is Murray reaction. Different functional moieties with thiol group (different color) can be swapped in place-exchange reaction which finally lead to step 3 involving further addition of different thiol ligand [34].

There are new studies demonstrated that PEGylated gold NPs ranging from 10-30 nm in diameter unable to cross human placenta within 6 hours, which imply that the drug could be restricted to just the mother and preventing teratogenic effect to the fetus [58].

Gold NPs still in its infancy. Comparing to anticancer studies, more progress have been made in DNA delivery for gene therapy [59] and in imaging applications [60]. Gold NPs could be functionalized with anticancer drugs such as paclitaxel and 6-mercaptopurine (6-MP) have been use with gold NPs [61]. It had been shown that Gold NPs enhanced paclitaxel anti-proliferation effects on tumor by cell adhesion disruption [62]. It has been

observed that anti-leukemia 6-MP bound to Gold NPs exhibit greater toxicity against leukemia compared to free 6-MP.

It has been reported that intracellular glutathione has been used with Gold NPs as a trigger for drug release [63]. Higher glutathione in cancerous cells and precancerous cells has been exploited in targeting intracellular release of chemotherapy drugs [64].

Albumin NPs:

Albumin NPs is one among other NPs that have been extensively investigated as a drug carrier which showed promising future. With molecular weight of 66 kDa, it is soluble in both water and ethanol, two favorited solvent for intravenous administration. Albumin NPs is non-toxic and tolerated by our immune system as albumin is presence in our circulating plasma. Its long half-life is particularly captivating as a drug carrier which is suitable for passive targeting [65].

To synthesize albumin NPs, albumin can be derived from blood product and human plasma and as alternative it can be produced from genetically engineered yeast cells [65]. Preparation of albumin NPs involve desolvation or coacervation process.

First drug based on albumin NPs is Abraxane®. Chemotherapy drug, paclitaxel, bound to 130 nm human albumin NPs. The advantage of Abraxane® over free paclitaxel is albumin NPs- paclitaxel have longer circulation half-life and lack of hypersensitivity-inducing CremophorEL® solvent [66]. The efficacy of Abraxane® has been confirmed in clinical trials thus is used routinely. Abraxane® is currently investigated with other taxanes for treatment of refractory prostate cancer by Haley and Frenkel (2008) which speculated that Abraxane® is transported into tumor cells by secreted protein acidic rich in cysteine or osteonectin [67].

Studies involving drug delivery into the brain also on-going using albumin NPs. Albumin-PEG- PLA Nps cross the blood-brain barrier and conjugation with apolipoprotein could facilitate transcytosis [68]. This is new avenue for the use of albumin NPs for drug delivery to the brain, not only limited to application in cancer treatments.

As a conclusion, It is important that the pharmaceutical compounds safely arrive to the intended destination with its desired therapeutic effect as expected. Liposomes and NPs show promising hope in this regard. Attractive properties possess by liposomes and NPs such as biocompatibility, low clearance rates, low toxicity and capability to target specific tissues and controlled release of the drugs is an added advantage compared to other method of drug deliveries. This drug delivery technology is not yet fully used as its need more studies to be done such as its toxicological long-term exposure and the refine reproducibility of the production of liposomes and NPs.

Peptides Applications in Cancer Targeted Therapy

Peptides are shorts linear amino acid chain (AA). Most of the AA are <50 AA in length with disulfide bonds [69]. Clinically relevant peptides can be designed and synthesized according to established methods with extremely high specificity to receptors that are over-expressed by several types of cancer cells. Interaction of these peptides-receptors provides promising approach for the development of diagnosis and therapy of anti-cancer agent [70]. Peptides have several advantages over monoclonal antibodies because they are much smaller in size, easy to synthesize and modulate, high specificity and affinity, high cell membrane penetration and low immunogenic effects. In addition, using peptide in cancer treatment can avoid accumulation of these administrated peptides in kidney or liver which can minimize their toxicity to the body [71].

Therapeutic peptides show great potential in cancer management. It can be used to bind with cytotoxic drugs, vaccines, hormones and radionuclides for cancer targeted therapy. Currently, more than 150 peptides are in clinical investigation and several of them showing highly competitive results [72]. Existing therapeutic peptides have been divided into three groups: antimicrobiol/pore forming peptides, cell-permeable peptides and tumor targeted peptides [73]. Tumor targeted peptides targets tumor-expressed receptor on the cell membrane. These peptides could be developed as a drug delivery system by attaching therapeutic drugs and deliver them to target tissues more precisely with a controlled release, and to be internalized into cancer cells to induce cell

death. Table 4 shows some of the examples of clinically relevant therapeutic peptides and their current development stages.

Table 4: Peptides and Its Potential Application in Cancer Diagnosis and Therapy [74]

Peptide	In vitro or/and in vivo validation study	Target
RGD-SSL-Dox	Melanoma (A375) and murine (B16-F10)melanoma cells	Tumor targeting
PNC-2 and PNC-7	Pancreatic cancer (MIA-PaPa) cells	Transduction pathway
Cardiac Natriuretic peptides	Pancreatic carcinoma (HPAC), Renal carcinoma (SW156), breast carcinoma (HCC1428), ovarian carcinoma (NIHOVCAR-3), modulady thyroid carcinoma (TT) glioblastoma carcinoma (LNZTA3WT4) and lung carcinoma	Transduction pathway
P16	Pancreatic cancer (AsPC-1 and BxPC-3) cells	Cell cycle
Bac-7-ELP-p21	Ovarian carcinoma (SKOV-3) cells	Cell cycle
R8-Bax	Cervical carcinoma (Hela) and murine mammary carcinoma (HCT-116) cells	cell death (apoptosis) induction
CT20p-NP	Breast cancer (MCF-7 or MDA-MB-231)and colon cancer (HCT-116) cells	Cell death (apoptosis) induction
PNC-28	Breast cancer (MDA-MB-453), colon cancer (HI299 and SW1417), osteosarcoma (SAOS2), cervical carcinoma (Hela) and pancreatic cancer (MiaPaCa-2) cells. Rat kras-transformed pancreatic cancer (TUC-3) and transformed endothelial (E49)cells	Tumor suppressor protein
Tat-aHDM2	Melanoma (MM-23 and MM-26), retinoblastoma (Y79 and WERI), osteosarcoma (U2OS), and cervical carcinoma cells	Tumor suppressor protein
TCP-1	Colorectal cancer	

One of the major challenges to the successful application of therapeutic peptides for cancer therapy is the relatively low in vivo stability of peptides to protease digestion in biological environments and fast renal clearance due to their small size. To overcome this problem, several approaches have been developed and studied. One of the most promising methods is to conjugate or incorporate them with non-biological materials such as nanoparticles (PNCs). A large number of studies have demonstrated that this method improved overall pharmacokinetic and pharmacodynamic properties of the peptide-conjugated nanoparitcles in vivo [75,76]. Another advantage of PNC is their ability to enhance the target-to-background for imaging study.

Over the past several decades, nanomedicine and nano-based drug delivery system have emerged into a promising tool in the delivering of cancer therapeutic agents. For example, nanoparticle-based methods have been developed for various application including treatment and imaging of cancer. NP can improve the efficacy of cytotoxic drug through several mechanisms such as selectively deliver the drug into solid tumors [74]. Conjugated targeting peptides on different types of NPs have been developed by various researchers to provide more effective drug delivery systems. Conjugation of different types of peptides, along with therapeutic agents, these PNCs can be engineered to target specific tumor or organ of interest. Encapsulated therapeutic agents not only are more stable in vivo, but also its circulation time in the body to be increased and maximized therapeutic effects [77].

A recent study investigated the mechanism of NP conjugated poly(glycidyl methacrylate) peptide targeting Engrailed 1, a transcription factor for basal breast cancer proliferation and metastasis. The peptide conjugated NP was loaded with docetaxel in the internal void of NP. The studies demonstrated that peptide-NP-docetaxel combination induced higher percentage of apoptosis on cancer cells as compared to either peptide or docetaxel alone [78]. Peptides has gained more important role in colorectal cancer diagnosis and treatment. It was reported elsewhere that, human neutrophil peptides 1-3 (HNP1-3) are highly expressed on CRC tissue. Other

than that, it was also found that high concentration of serum C-peptide was present in most of male's adenoma cases [79]. These results suggest that those peptides can be developed as marker to detect CRC in early stage. As summarized above, peptides are important and have high potential to be developed as new cancer targeting agent. The advanced method in incorporating nanoparticles with peptides has enhanced the in vivo stability, pharmacokinetic and pharmacodymanic properties of these PNCs. However, there are several challenges which need to be addressed in order to translate these peptides in clinical. First, the in vivo behaviour of these PNCs has to be fully studied. In addition, the stability of peptide conjugated with nanoparticles in vivo needs to be improved [80]. Furthermore, the process to conjugate peptide and nanoparticles could affect or change the biological properties of peptides. Therefore, more studies need to be carried out in order to address those obstacles and make the PNCs application into routine clinical practice become possible.

In-vitro anti-cancer activity of nanoparticles, peptides and peptide-conjugated nanoparticles

Many nanoparticles have been used for conjugation with peptides for clinical applications such as metallic nanoparticles, magnetic nanoparticles and protein (albumin) nanoparticles. Peptides used for conjugation with nanoparticles in many studies are either newly produced or commercially available [81-83]. Anti-cancer study of nanoparticles (NPs), peptides and peptide- conjugated NPs were well studied and scientifically proven. In-vitro cytotoxic evaluations were performed in model cancer cell line such as HT29 and DLD-1 which represent colon cancer cells, MDA-MB-231 which represent breast cancer cells, BxPC-3 which represent pancreatic tumor cells and etc. Apoptosis induction and reactive oxygen species (ROS) production analysis were normally performed to determine the cytotoxic mechanisms of NPs, peptides and peptide-conjugated NPs. Cellular uptake of these substances was also normally elucidated to determine internalization ability. Two common characteristics of a peptides-conjugated NPs to be used as a system for delivering anti-cancer agents are the higher degree of cytotoxicity and the ability to internalize cancer cells [81, 82, 84].

The use and application of metallic nanoparticles in biomedical field was increased in recent years. In cancer biology, metallic nanoparticles are widely used for the delivery and targeting of pharmaceutical, therapeutic and diagnostic agents. The metallic nanoparticles can be synthesized as required and further modified for conjugation with biological ligands and anticancer drugs. The multifunctional nanoparticles constructed currently are supposed to show superior effects on cancer cells [84, 85]. Aswathanarayan et al, (2018), found that metallic nanoparticles (NPs) such as gold (AuNPs), iron oxide (Fe_2O_3 NPs) and zinc oxide (ZnONPs) possess cytotoxic effect against colon cancer cell line, HT29 [81]. Among the three NPs, ZnONPs showed the highest and significant cytotoxic activity with IC_{50} value of 17.12 p,g/ml. Cellular morphology analysis showed that ZnONPs inhibited HT29 cell growth. Fluorescence microscopic analysis showed that cytotoxic mechanism of ZnONPs against HT29 cells was through apoptosis induction. ZnONPs induced apoptosis event in a concentration dependent manner (0, 20 and 50 p,g/ml). Another cytotoxic mechanism of ZnONPs in HT29 cells was the increment of reactive oxygen species (ROS) production that was determined through dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. Like apoptosis, ZnONPs also increase ROS production in a concentration dependent manner (0.5 to 50.0 p,g/ml). Inside the cells, ZnONPs act as a redox system and react with chemical entities in the cell and generate high amount of ROS, leading to oxidative stress. Higher concentration of intracellular ROS can cause damage to lipid, protein, nucleic acid, membranes and organelles in the cells, which can lead to activation of cell death processes such as necrosis and apoptosis [86, 87]. ZnONPs was then conjugated with two novel hydrophobic peptides namely Boc-Leu-Aib-Val-dPro-lPro-Val-Aib- Leu-OMe (peptide-1) and Boc-U-Gpn-OMe (Peptide-2). The resulted conjugated products (peptide 1-ZnONPs and peptide 2-ZnONPs complexes, were tested for their cytotoxic activity against HT29 cells. ZnONPs, peptide-1 and peptide-2 alone were also tested. The results exhibited that both peptides-ZnONPs complexes inhibited HT29 cell growth in a concentrations a dependent manner (0.5, 5.0 and 10.0 p,g/ml). Interestingly, the degree of cell growth inhibition activity of both peptides-ZnONPs complex was higher than that of peptides and ZnONPs alone. This result showed that conjugation of hydrophobic peptides with ZnONPs produce higher cytotoxic activity compared to NPs and peptides alone. This study showed that the hydrophobic peptides-ZnONPs conjugates may have the potential to be developed as new system for delivering anti-cancer drugs particularly to colon cancer. Further studies need to be carried out to determine cellular uptake ability of the conjugates by the targeted cells [81].

Another study done by Banerjee et al., (2019) on metallic nanoparticles reported that peptide- silver nanoparticles (AgNPs) and peptide-gold nanoparticles (AuNPs) conjugates exhibited strong cytotoxicity and apoptosis induction activity against colon (HT29) and breast cancer cells (MDA-MB-231) [84]. Initially, Banerjee and co-worker synthesized five new peptides namely peptide-1 (Boc-L-^{D}P-L-OMe), peptide-2 ($(U^{B}F)_4$-OMe), peptide-3 (Boc-P-A-OH), peptide-4 (Boc-P-U-OH) and peptide-5 (Boc-pGly-pGly-OMe). All of these peptides were then screened for cytotoxicity against both HT29 and MDA-MB-231cells. The results showed that among all peptides, peptide-1 possesses the highest cytotoxicity against HT29 cells at all concentrations tested (1-20 p,M) and thus peptide-1 was chosen for conjugation with AgNPs and AuNPs. Both conjugates were characterized and subjected for further cytotoxic evaluation. In HT29 cells, peptide-1-AgNPs conjugate exhibited strong cytotoxic activity with the percentage of cell growth inhibition was 79% compared to peptide-1 alone (19%) and AgNPs alone (61%). In MDA-MB- 231 cells, peptide-1-AgNPs conjugate exhibited stronger cytotoxic activity with the percentage of inhibition was 93% compared to peptide-1 alone (29%) and AgNPs alone (70%). The similar trend was seen with peptide-1-AuNPs conjugate, where it showed cytotoxic activity higher than the peptide-1 alone or AuNPs alone, in both HT29 and MDA-MB-231cells. In HT29 cells, peptide-1-AuNPs exhibited cell growth inhibition up to -80% and in MDA-MB-231cells, the conjugate illustrated cell growth inhibition up to -97%. Fluorescent imaging analysis using nucleic acid-binding dyes; propidium iodide (PI) and acridine orange (AO) revealed that peptide-1, AgNPs, AuNPs, peptide-1-AgNPs conjugate and peptide-1-AuNPs conjugate induced apoptosis in both type of cells. This can be concluded through swelling and damages of the cells as well as condensation and structure deformation of the cells. The degrees of apoptosis event induced by both of peptide-1-NPs conjugate were higher compared to the peptide-1 or NPs alone. DNA fragmentation analysis was carried out to further confirmed the occurrence of apoptosis event in both cancer cells following treated with peptide-1, AgNPs, AuNPs, peptide- 1-AgNPs conjugate and peptide-1-AuNPs conjugate. One of the hallmark features of apoptosis is the cleavage of the DNA into oligonucleosomal fragments represented by multiples base pair of nucleotides (<200 bp). Visualizing these fragments can aid in characterizing an apoptotic event [88]. Treatment of both HT29 and MDA-MB-231cells with peptide-1, AgNPs, AuNPs, peptide-1-AgNPs conjugate and peptide-1-AuNPs conjugate resulted in degradation of DNA into small oligonucleosomal fragments, indicated occurrence of apoptosis process in both HT29 and MDA-MB-231cells [84].

Kalimuthu et al., (2018) found that metallic nanoparticles such as AuNPs can prolong the half-live of certain peptide in body fluid [89]. Initially, Kalimuthu and his group synthesized eight peptides using solid phase method. These peptides were then tested for their ability to internalize A20 murin lymphoma cells. Out of eight peptides, only peptide-4 and peptide-8 can internalize the target cells and localized at extra-nuclear area. Peptide-4 was then chosen for conjugation with cancer drugs namely chlorambucil, melphalan and bendamustine. All the three peptide-4-drug conjugates (PDCs) were tested for their cytotoxic activity against A20 cells and the results showed that all PDCs inhibited A20 cells growth in a dose dependent manner (10-50 pM). At highest dose, cell growth inhibition was more than 70%. Despite the ability to internalize cells and inhibit cells growth, PDCs have one drawback that is short half-live in biological fluids ranging from 10.60 to 15.40 min, which in turns may limiting the clinical applications of these PDCs. Kalimuthu and his group hypothesized that the PDCs, when conjugated to AuNPs may improve the PDCs bioavailability. AuNPs was chosen in their study because it has longer plasma half-life and can stabilize the PDCs. Prior to conjugation with PDCs, the AuNPS were initially coated with PEG to facilitate the non-covalent loading of PDCs. The complex PDCs-PEG-AuNPs were then tested for their bioavailability and cytotoxic activity against A20 cells. The results showed that the plasma half-lives of complex PDCs-PEG-AuNPs were extended to 21.0 to 22.3 hours. The PDCs-PEG-AuNPs retained cytotoxicity towards the target cells and such activity remain consistent even after 72-hours incubation. This study suggests that limitation of the short half-live of the PDCs in biological fluids and tissue can be overcome by conjugating them to PEG-AuNPs. By significantly extending PDCs stability, this conjugation also opens up the possibility of developing slow-release formulations of targeted drug delivery systems containing PDCs.

Niemirowicz et al., (2015) hypothesized that the anti-cancer activity of cathelicidin LL-37 (LL-37) peptide and ceragenin CSA-13 (CSA-13) can be improved when attached to the surface of magnetic nanoparticles (MNPs) [81]. To prove the hypothesis, they evaluate cell viability and apoptosis induction activity of LL-37, CSA-13,

MNPs, MNPs-LL-37 complex and MNPs-CSA-13 complex in two colon cancer cell lines, DLD-1 and HT-29 cells. In DLD-1 cells, MNPs and LL-37 peptide alone did not cause any effect on cell viability and cell apoptosis program. In contrast, MNPs-LL-37 complex exhibited a dose-dependent cell viability decrease activity (<50% at concentration of 100 ^g/ml), and moderate apoptosis induction activity (<50% at concentration of 100 ^g/ml). MNPs-LL-37 complex also reduced DLD-1 cells proliferation markedly at higher concentrations (proliferation rate <40%). Unlike the LL-37, CSA-13 alone produced a significant dose dose-dependent cell viability decrease activity. MNPs-CSA-13 complexes also showed similar trend of activity. At higher concentrations, both CSA-13 and MNPs-CSA-13 complex showed tremendous cell viability decrease activity with the percentage of viable cell was <20%. Both CSA-13 alone and MNPs-CSA-13 complex also induce apoptosis in DLD-1 cells significantly and in a dose dependent manner. Interestingly, apoptosis induction activity of CSA-13 alone was significantly higher than that of MNPs-CSA-13 complex. In HT-29 colon cancer cells, MNPs possess moderate cell viability decrease activity (-50%) and this activity was higher than LL-37 and MNPs-LL-37 complex. In apoptosis study, however, the MNPs-LL-37 complex showed moderate activity (-30%) but higher than MNPs and LL-37. Both peptide and complex reduced HT-29 cells proliferation weakly (proliferation rate >80%). CSA-13 and MNPs-CSA-13 complex showed excellent cell viability decrease activity against HT-29 cells (viable cells <50%). However, in apoptosis induction study, the CSA-13 alone showed higher activity (-85%) than the MNPs-CSA-13 complex. Both CSA-13 and MNPs-CSA-13 complex reduced proliferation of HT- 29 cells moderately (proliferation rate >60%). Internalization of LL-37 and CSA-13, MNPs-LL-37 and MNPs-CSA-13 complexes into HT-29 cells was assessed microscopically by tagging the peptides and MNPs with fluorescent dyes and added into HT-29 cells. Results showed that MNPs-LL-37 and MNPs-CSA-13 complexes were successfully internalized HT-29 cells. The data indicated that the MNPs accumulated in the cytoplasm while CSA-13 and LL-37 peptides were present in nucleus suggested that both peptides might play a key role as novel homing molecules. This study showed that combination of LL-37 and CSA-13 peptides with MNPs decreases viability and also induces apoptosis in HT29 cells. MNPs-LL-37 and MNPs-CSA-13 might have the potential to be developed as targeted anti-cancer agents for colorectal cancer due to its cytotoxic activity and also its ability to internalize HT-29 cells [82].

Yu et al., (2016) reported that gemcitabine-loaded human serum albumin nanoparticles (Gem- HSA-NPs) exhibited moderate cytotoxicity against human pancreatic cancer cell lines, BxPC-3 [83]. Then, they enhance pancreatic cancer targeting by conjugating cyclic arginine-glycine- aspartic (cRDG) peptide to the surface of Gem-HSA-NPs. The complex cRDG-Gem-HSA-NPs were formulated through nanoparticle albumin-bound (nab) technology. The resulting complex was subjected to cytotoxic analysis against BxPC-3 cells. They found that cRDG-Gem-HSA- NPs complex inhibited the growth of BxPC-3 cells in a dose dependent manner and possess strong cytotoxic activity with the IC_{50} value was 0.1 pg/ml. This cytotoxicity activity was higher compared to gemcitabine alone (IC_{50} value 0.28 p,g/ml) and Gem-HSA-NPs alone (IC_{50} value

42 p,g/ml). This study showed that cRDG-anchored NPs can deliver gemcitabine to pancreatic cells more efficient as compared to free drug and also non-targeted NPs. Such findings make the cRDG-Gem-HSA-NPs suitable to deliver anti-cancer drugs to pancreatic tumor cells. However, further studies need to be carried out to determine cellular uptake or internalization ability of the cRDG-Gem-HSA-NPs complex into the targeted cells [83]. These in vitro studies suggest that conjugating nanoparticles with certain peptides increased selectivity and cytotoxicity against cancer cells as well as improved cellular uptake or over the conventional chemotherapeutic drugs and non-targeted nanoparticles, which in turn will enhanced drug's efficacy and safety. However, in vivo pre-clinical studies as well as clinical studies are required to be carried out in order to confirm these findings.

CANCER DIAGNOSTIC IMAGING

Cancer Imaging Basics

Cancer may be difficult to detect, but for some types of cancer, the earlier it is detected, the better are the chances of treating it effectively. Imaging techniques, methods of producing pictures of the body have become an important element of early detection for many cancers. But imaging is not simply used for detection.

Imaging is also important for determining the stage (telling how advanced the cancer is) and the precise locations of cancer to aid in directing surgery and other cancer treatments, or to check if a cancer has returned. Clinical trials, research studies involving people, play an essential role in determining whether emerging imaging techniques are effective and safe [90].

There is no single imaging test that can accurately diagnose cancer. A complete evaluation usually requires a detailed health history and physical exam along with diagnostic testing. Many tests are needed to determine if a person has cancer, or if another condition (such as an infection) is mimicking the symptoms of cancer. Effective diagnostic testing is used to confirm or eliminate the presence of disease, monitor the disease process, and plan for and evaluate the effectiveness of treatment. Tests may be done for staging, to determine the extent of cancer and if it has spread. Or tests may be done to assess prognosis or select specific therapies. In some cases, repeat testing is needed when a person's condition has changed, or if a sample collected was not of good quality, or an abnormal test result needs to be confirmed. Diagnostic procedures for cancer may include imaging, lab tests (including tests for tumour markers), tumour biopsy, endoscopic exam, surgery, or genetic testing. Tests are often repeated regularly throughout treatment to determine the treatment's effectiveness of treatment and the cancer's response to it. This is called restaging. A cancer diagnosis requires a biopsy or analysis of involved tissue or blood. An imaging test cannot be used to diagnose cancer [91].

Types of diagnostic imaging

Imaging is the process of producing valuable pictures of body structures and organs. It is used to find or detect tumours and other abnormalities, determine the extent of disease, and evaluate the effectiveness of treatment. Imaging may also be used when doing biopsies and other surgical procedures. There are various types of imaging tests that may be used for diagnostic imaging, namely, X-ray, Computed Tomography (CT) scan, Mammogram, Ultrasound, Magnetic Resonance Imaging (MRI) and Nuclear Medicine scan (PET/CT, PET/MRI, SPECT/CT, SPECT/MRI)

X-ray

X-rays are diagnostic tests that use invisible electromagnetic energy beams to produce images of internal tissues, bones, and organs on film. X-rays may be taken of any part of the body to detect a tumour or cancer.

Computerized Tomography (CT) scan

A non-invasive diagnostic imaging procedure that uses a combination of X-rays and computer technology to produce both horizontal, and axial, images (often called slices) of the body. A CT scan shows detailed images of any part of the body, including the bones, muscles, fat, and organs. CT scans are more detailed than general X-rays.

Mammogram

A mammogram is an X-ray exam of the breast. It is used to detect and diagnose breast disease in women who have breast problems such as a lump, pain, or nipple discharge. It is also used for women who have no breast complaints. Mammography can't prove that an abnormal area is cancerous. But if it raises a significant suspicion of cancer, a biopsy must be done to confirm a cancer diagnosis. During a biopsy procedure, a tissue sample is removed by needle or open surgical biopsy. Then the tissue is checked under a microscope to see if it is cancer. Mammography has been used for about 30 years. In the past 15 years technical advancements have greatly improved both the technique and results. Today, special equipment, used only for breast X-rays, produces studies that are high in quality but low in radiation dose. Radiation risks are considered to be very minor.

Ultrasound (sonography)

This diagnostic procedure uses high-frequency sound waves and a computer to create images, called sonograms, of blood vessels, tissues, and organs. Sonograms are used to view internal

organs as they function and to assess blood flow through various vessels. Tumours in the belly (abdomen), liver, and kidneys can often be seen with an ultrasound. (Ultrasound is not useful in the chest because the ribs

block the sound waves.) Ultrasound can be used through a probe that can be inserted into organs, such as the anus, vagina, or esophagus and brought closer to the internal organs, producing a more accurate picture.

Magnetic Resonance Imaging (MRI)

MRI is a diagnostic procedure that uses a combination of a large magnet, radiofrequencies, and a computer to produce detailed images of organs and structures within the body. An MRI is often used to examine the heart, brain, liver, pancreas, male and female reproductive organs, and other soft tissues. It can assess blood flow, detect tumors and diagnose many forms of cancer, evaluate infections, and assess injuries to bones and joints.

Nuclear medicine scan

Nuclear medicine scan is a method of producing images by detecting radiation from different parts of the body after a radioactive tracer is given to the patient. The images are digitally generated on a computer and transferred to a nuclear medicine physician, who interprets the images to make a diagnosis.

Some common nuclear scans are SPECT scan and PET scan. Nuclear scans make pictures based on the body's chemistry (like metabolism) rather than on physical shapes and forms (as is the case with other imaging tests). These scans use liquid substances called radionuclides (also called tracers or radiopharmaceuticals) that release low levels of radiation. Body tissues affected by certain diseases, such as cancer, may absorb more or less of the tracer than normal tissues.

Special cameras pick up the pattern of radioactivity to create pictures that show where the tracer travels and where it collects. If cancer is present, the tumor may show up on the picture as a "hot spot" - an area of increased cell activity and tracer uptake. Depending on the type of scan done, the tumor might instead be a "cold spot" - a site of decreased uptake (and less cell activity). Nuclear scans may not find very small tumors and cannot always tell whether a tumor is really cancer. These scans can show some internal organ and tissue problems better than other imaging tests, but they don't provide very detailed images on their own. Because of this, they are often used along with other imaging tests to give a more complete picture of what's going on. For instance, bone scans that show hot spots on the skeleton are usually followed by x-rays of the affected bones, which are better at showing details of the bone structure. Some nuclear scans are also used to measure heart function [92].

Gamma Camera vs Single Photo Emission Computed Tomography (SPECT) scan

SPECT/CT and SPECT/MRI combine two imaging technologies - SPECT which shows biological functions in the body, and Computerized Tomography (CT) or Magnetic Resonance (MRI), which shows detailed anatomical structures. If a scanner does not have the CT or MRI components, it is more commonly known as a gamma camera rather than a SPECT scanner. The gamma camera produces image in 2D whereas the SPECT, SPECT/CT or SPECT/MRI cameras are able to produce a 3D image. A SPECT/CT or SPECT/MRI scan allows your doctor to see how your organs function and to detect certain diseases. It is useful for a range of medical conditions, such as bone metastases, renal function, cardiac blood flow, gastrointestinal function, immune activity, thyroid function, tumours and more. The radioisotopes commonly used for this procedure are Tc-99m, I-123, I-131, In-111, Sm-153, Lu-177, Re-188, Re-186 etc.

Positron emission tomography (PET) scan

PET is a specialized procedure used to examine various body tissues to identify certain conditions. PET may also be used to follow the progress of the treatment of certain conditions. For example, PET studies evaluate the metabolism (utilization of tagged glucose molecules; [18F]FDG) of a particular organ or tissue, so that information about the physiology (functionality) and anatomy (structure) of the organ or tissue is evaluated, as well as its biochemical properties. Thus, PET may find biochemical changes in an organ or tissue. These changes can identify the start of a disease process before anatomical changes related to the disease can be seen with other imaging processes, such as CT or MRI. More recently, PET/CT does PET and CT at the same time and produces a composite image that can both produce a picture of an organ and measure its use of sugar [93].

A PET/MRI scan is a two-in-one test that combines images from a positron emission tomography (PET) scan and a magnetic resonance imaging (MRI) scan. This new hybrid technology harnesses the strengths of PET and MRI to produce some of the most highly detailed pictures of the inside of your body currently available. Doctors use those pictures to diagnose medical conditions and plan their treatment. For example, PET/MRI scans of the brain are useful in the care of Alzheimer's disease, epilepsy, and brain tumors. The radioisotopes commonly used for PET procedures are F-18, C-11, N-13, O-15, I-124, Cu-64, Zr-89 etc.

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