Stomach cancer or gastric cancer (GC) refers to cancer arising from any part of the stomach. GC arises from inflammation and is preceded by a lengthy precancerous process, developing via multiple sequential steps [Orlando. 2002]. GC ranks fourth in terms of prevalence and second in terms of mortality with a projected 6, 50,000 deaths annually [Zhang et al. 2004]. GCs’ are highly heterogeneous with distinct pathological patterns and clinical behaviors [Ooi et al. 2009]. Most cases of GC are diagnosed at late stages with a five year survival rate of 24%. However, if GC is diagnosed at early stages, the five-year survival rate is about 61% [Cancer facts & Figures. 2008]. Lauren’s classification is the traditional method for classifying GCs’ into histological subtypes according to the structural features, histological appearances of the cells, and the level of mucus [Lauren. 1965]. The prognosis of GC depends heavily on tumor stage at diagnosis. Surgical resection, still the mainstay of treatment, is very effective in early stage cancer. Most of the GC patients are diagnosed in advanced stages, when the prognosis is extremely poor. Targeted therapies against deregulated pathways using antibodies and signaling pathway inhibitors provides novel opportunities in the treatment of GC along with the combinational chemotherapy [Tabernero et al. 2005]. Recent studies have shown that comprehensive analysis of gene expression patterns can identify new molecular criteria for classification and prognostication of diverse cancers [Cunningham et al. 2006]. Therefore, there is a critical need for the identification of new molecular classification/ diagnostic system, therapeutic targets and chemotherapeutics for GC therapeutics.

Cellular signaling pathways are the biochemical events which drives cellular processes. If there is an alteration or modulation leads to disease. Wnt signaling pathway involves in developmental process. Dysregulated Wnt signaling pathway leads to gastrointestinal tumors. The Wnt signaling pathway, named for its most
upstream ligands, the Wnts, is involved in various differentiation events during embryonic development and leads to tumor formation when aberrantly activated. Molecular studies have pinpointed activating mutations of the Wnt signaling pathway as the cause of approximately 90% of gastric and colorectal cancer and somewhat less frequently in cancers at other sites, such as hepatocellular carcinoma (HCC).

Numerous studies suggest that activation of the Wnt/β-catenin signaling pathway plays an important role in tumorigenesis of GC [Pan et al. 2008; Dvory et al. 2007; Evert et al. 2003]. Nuclear β-catenin is the hallmark of an active canonical Wnt pathway. A gain or loss-of-function mutations of several members in this pathway, such as β-catenin, AXIN or APC, have been found in many types of human tumors. The identification of similar important regulatory factors offers an opportunity to develop new therapies targeting this pathway at the extracellular/membrane, cytoplasmic, and nuclear levels [Luu et al. 2004].

**Schematic representation of Wnt/β-catenin signaling pathway**

![Wnt/β-catenin signaling pathway diagram](image-url)
A comprehensive picture of \textit{Wnt} regulated genes in GC remains unexplored. It is necessary to explore and identify/catalogue all \textit{Wnt} regulated genes in GC. Understanding of frequent changes in gene expression in \textit{Wnt} signaling at the molecular level in GC may help to develop novel molecular diagnostic strategies and targeted therapies which in-turn will improve the treatment of this disease. Identification of \textit{Wnt} targets in a comprehensive manner will pave way to identify diagnostic biomarker candidates, which can identify GCs' with dysregulated \textit{Wnt} signaling pathway. With this approach, it is possible to identify and target a component of the \textit{Wnt} signaling pathway in GC. Investigation of \textit{Wnt} signaling pathway in molecular genomic level is meaningful in the context of GC therapeutic identifications.

Therefore the present work is aimed to carry out on the following aspects.

1. \textbf{To delineate the \textit{Wnt}/\textit{β-catenin} mediated transcriptional changes in gastric cancer cell lines.}
2. \textbf{To screen the expression pattern \textit{Wnt} regulated genes in primary gastric tumors.}
3. \textbf{To identify the robust genes or gene sets for gastric cancer diagnostics.}
1. Gastric Cancer

Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the second most common cause of cancer related death worldwide [Parkin et al. 2004; Ferlay et al. 2007]. Although the incidence of GC has gradually decreased over the last half century, cancer at proximal stomach is on the rise [Devasa et al. 1998; Blot et al. 1991]. Today, GC is still the seventh most common cause of cancer-related death in the United States [Jemal et al. 2011] and the prognosis of advanced GC remains poor. Gastric carcinogenesis is a multistep and multifactorial process. While the intestinal type of GC is often related to environmental factors such as *H. Pylori* infection, diet, and life style, the diffuse type is more often associated with genetic abnormalities.

1.1. Gastric Cancer Incidence and Mortality Worldwide

About one million new cases of stomach cancer were occurred in 2008 (988 000 cases, 7.8% of the total), making it currently the fourth most common malignancy in the world, behind cancers of the lung, breast and colo-rectum. More than 70% of cases (713 000 cases) occur in developing countries (467 000 in men, 246 000 in women), and half the world total occurs in Eastern Asia (mainly in China). Age-standardized incidence rates are about twice as high in men as in women, ranging from 3.9 in Northern Africa to 42.4 in Eastern Asia for men, and from 2.2 in Southern Africa to 18.3 in Eastern Asia for women [Cancer facts & Figures. 2008].

Stomach cancer is the second leading cause of cancer death in both sexes worldwide (736 000 deaths, 9.7% of the total). The highest mortality rates are estimated in Eastern Asia (28.1 per 100,000 in men, 13.0 per 100,000 in women), the lowest in Northern America (2.8 and 1.5 respectively). High mortality
rates are also present in both sexes in Central and Eastern Europe, and in Central and South America [Cancer facts & Figures. 2008].

International variation in age-standardized stomach cancer incidence rates among males

International variation in age-standardized stomach cancer incidence rates among females
1.2. Histologic classification of gastric carcinomas

Over the past half century the histologic classification of gastric carcinoma has been largely based on Lauren’s criteria, in which intestinal type and diffuse type adenocarcinoma are the two major histologic subtypes, plus indeterminate type as uncommon variant [Lauren. 1965]. The relative frequencies are approximately 54% for intestinal type, 32% for the diffuse type, and 15% for the indeterminate type [Hwang et al. 2010]. There are indications that the diffuse type gastric carcinoma is more often seen in female and young individuals, while the intestinal type adenocarcinoma is more often associated with intestinal metaplasia and H. Pylori infection [Kanoke et al. 2001; Parsonnet et al. 1991].

The 2010 WHO classification recognizes four major histologic patterns of GCs': tubular, papillary, mucinous and poorly cohesive (including signet ring cell carcinoma), plus uncommon histologic variants. The classification is based on the predominant histologic pattern of the carcinoma which often co-exists with less dominant elements of other histologic patterns.

Tubular adenocarcinoma is the most common histologic type of early gastric carcinoma. It tends to form polypoid or fungating masses grossly, and histologically demonstrates irregularly distended, fused or branching tubules of various sizes, often with intra luminal mucus, nuclear and inflammatory debris.

Papillary adenocarcinoma is another common histologic variant often seen in early gastric carcinoma. It tends to affect older people, occur in the proximal stomach, and is frequently associated with liver metastasis and a higher rate of lymph node involvement. Histologically, it is characterized by epithelial projections scaffolded by a central fibrovascular core.

Mucinous adenocarcinoma accounts for 10% of gastric carcinoma. Histologically it is characterized by extracellular mucinous pools which constitute at least 50% of tumor volume. The tumor cells can form glandular architecture and irregular cell clusters, with occasional scattered signet ring cells floating in the mucinous pools.
Signet ring cell carcinoma and other poorly cohesive carcinomas are often composed of a mixture of signet ring cells and non-signet ring cells. Poorly cohesive non-signet ring tumor cells are those that morphologically resemble histiocytes, lymphocytes, and plasma cells. Those tumor cells can form irregular microtubaculae or lace-like abortive glands, often accompanied by marked desmoplasia in the gastric wall and with a grossly depressed or ulcerated surface. When it occurs at the antropyloric region with serosal involvement, the carcinoma tends to have lymphovascular invasion and lymph node metastasis. Because signet ring cell and other poorly cohesive carcinomas at antropyloric region have a propensity to invade duodenum via submucosal and subserosal routes including subserosal and submucosal lymphatic spaces, special attention needs to be paid to those routes when a distal margin frozen section is requested at the time of surgical resection. Special stains such as cytokeratin immunohistochemistry can help detect morphologically occult signet ring cells in the lamina propria. One important differential diagnosis of neoplastic signet ring cells in gastric mucosa is benign pseudo-signet ring cells which can remarkably mimic signet ring cell carcinoma [Binghu et al. 2012].

1.3. Risk Factors for Gastric Cancer

There are multiple risk factors for the development of gastric adenocarcinoma, including precursor conditions, genetic and environmental conditions. Risk factors are: dysregulations of signaling processes, *H. Pylori* infection, A diet high in salty and smoked foods, A diet low in fruits and vegetables, Eating foods contaminated with aflatoxin fungus, Family history of stomach cancer, Infection with *H. Pylori*, Long-term stomach inflammation, Pernicious anemia, Smoking, Stomach polyps.
1.4. Symptoms of Gastric Cancer

Indigestion and stomach discomfort, A bloated feeling after eating, Mild nausea, Loss of appetite, Heartburn, Discomfort in the upper or middle part of the abdomen, Blood in the stool (which appears as black, tarry stools), Vomiting or vomiting blood, Weight loss, Pain or bloating in the stomach after eating, Weakness or fatigue associated with mild anemia (a deficiency in red blood cells).
1.5. Diagnostic methods for Gastric Cancer

The initial diagnosis of gastric carcinoma often is delayed because up to 80 percent of patients are asymptomatic during the early stages of stomach cancer. In Japan, a higher incidence of adenocarcinoma and rigorous screening processes have led to a greater number of cases of GC being detected in an early stage (i.e., when limited to the mucosa and sub mucosa, with or without lymph node involvement). Unfortunately, in the United States, most cases of GC are discovered only after local invasion has advanced.

Weight loss, abdominal pain, nausea and vomiting, early satiety, and peptic ulcer symptoms may accompany late-stage GC. Signs may include a palpably enlarged stomach, a primary mass (rare), an enlarged liver, Virchow’s node (i.e., Left supraclavicular), Sister Mary Joseph’s nodule (periumbilical), or Blumer’s shelf (metastatic tumor felt on rectal examination, with growth in the rectouterine/rectovesical space).

Patients presenting with the aforementioned symptoms and those with multiple risk factors for gastric carcinoma require further workup. Esophago gastroduodenoscopy (EGD) is the diagnostic imaging procedure of choice in the work-up of gastric carcinoma [Capell et al. 2002]. However, a double-contrast barium swallow, a cost-conscious, noninvasive, and readily available study, may be the initial step [Low et al. 1994]. This radiographic study provides preliminary information that may help the physician determine if a gastric lesion is present and whether the lesion has benign or malignant features. Gastric ulcers without any malignant characteristics seen on barium swallow have a specificity of more than 95 percent in ruling out GC. However, when indeterminate results are reported or when both benign and malignant signs are present, further diagnostic evaluation is necessary.

EGD is a highly sensitive and specific diagnostic test, especially when combined with endoscopic biopsy. Multiple biopsy specimens should be obtained from any
visually suspicious areas; this step involves repeated sampling at the same tissue site, so that each subsequent biopsy reaches deeper into the gastric wall.

After the initial diagnosis of GC is established, further evaluation for metastases is necessary to determine treatment options. Computed tomographic (CT) scanning is a useful method of detecting liver metastases greater than 5 mm in diameter, perigastric involvement, peritoneal seeding, and involvement of other peritoneal structures (e.g., ovaries, rectal shelf). However, CT scanning is unable to allow assessment of tumor spread to adjacent lymph nodes unless they are enlarged. In addition, it has not been shown to be effective in allowing determination of the depth of tumor invasion and cannot reliably support detection of solitary liver or lung metastases smaller than 5 mm in diameter [Ziegler et al. 1993].

Endoscopic ultrasonography (EUS) is a modality that allows for more accurate staging. In EUS, the transducer is placed directly next to the gastric wall, and high-frequency sound waves are used to determine the depth of tumor invasion and detect local lymph node involvement, which may be assessed by operative biopsy.

Random biopsies beyond lesion areas also are important in achieving a correct tissue diagnosis. The updated Sydney system [Dixon et al. 1996] recommends that at least five biopsy specimens be taken (two from the antrum within 2 to 3 cm of the pylorus, two from the corpus about 8 cm from the cardia, and one from the incisura angularis). Because tumor depth and lymph node involvement influence survival, EUS is an important tool for increasing preoperative staging accuracy. However, EUS cannot permit assessment of tissue beyond a depth of about 5 cm and, therefore, cannot be used to assess distant lymph node involvement or to screen for lung or liver metastases. Recent literature supports the combination of CT scanning and EUS for preoperative staging of GC to best determine the number and location of involved lymph nodes.
1.6. Treatment strategies for Gastric Cancer

1.6.1. Radiotherapy

Although smaller studies have shown some clinical response to radiotherapy (local-regional control) in patients with GC, only a modest survival advantage has been shown. A usual dosing regimen of radiation therapy is 45 to 50 Gy in 20 to 30 fractions. The adverse effects caused by radiation therapy include gastrointestinal toxicity from dose-limiting structures surrounding the stomach (intestines, liver, kidneys, spinal cord, and heart).

1.6.2. Chemotherapy

After several trials, significant survival advantage deriving from the use of chemotherapy as a definitive treatment for GC has not been reported. It is important to note, however, that one study [Allum et al. 1989] revealed recurrence rates of up to 80 percent in patients undergoing surgical resection alone, suggesting a need to continue investigation of adjuvant chemotherapy and radiotherapy.

1.6.3. Surgery

According to the recommendations of the International Union against Cancer and the Japanese Research Society for GC, GC is classified according to its location in the proximal, middle, or distal stomach [Siewert et al. 1997]. Although the borders between these thirds are not precisely defined, this definition has proved to be useful for determining the extent of resection. The selection of the surgical procedure in patients with GC should be primarily adjusted to the location of the tumor, the growth pattern seen on biopsy specimens, and the expected location of lymph node metastases. In patients with proximal-third GC, an extended gastrectomy, including the distal esophagus, is necessary. For distal-third GC, patients may be able to undergo subtotal gastrectomy if biopsy reveals “intestinal-type” adenocarcinoma. Total gastrectomy is recommended if the biopsy shows “diffuse-type” carcinoma. Middle-third GC always requires total gastrectomy. Current operative mortality rates are reported to be as low as 1 to 3
percent. The most common postoperative complication is tumor recurrence. Five-year survival rates for post resection early GC have been reported to be as high as 90 percent. However, survival rates significantly decrease according to tumor penetration and lymph node invasion [Hundhal et al. 2000] Because of the extensive lymphatic network of the stomach and the propensity for microscopic extension, the traditional surgical approach attempts to maintain a 5-cm margin proximally and distally to the primary lesion. Many studies report that nodal involvement indicates a poor prognosis, requiring the use of more aggressive surgical approaches to attempt to remove involved lymph nodes. However, the extent of lymph node resection remains a matter of controversy. Retrospective studies from Japan showed promising results of increased survival without increased operative morbidity and mortality when extended lymphadenectomy was performed [Siewert et al. 1998; Kaibara et al. 1990]. However, prospective follow up studies did not confirm these findings. In addition, some studies [Iriyama et al. 1989; Bonenkamp et al, 1999] have shown increased morbidity and mortality related to this extensive procedure.

1.6.4. Combination Approach

Although numerous randomized clinical trials have failed to show consistent survival benefits from adjuvant radiation therapy or chemotherapy alone in the treatment of GC, some studies have shown that patients receiving combined chemo radiation therapy have demonstrated improved disease free survival and improved overall survival rates. In one series patients were randomized to receive postoperative radiotherapy and 5-fluorouracil chemotherapy or surgery alone. Results of this study demonstrated improved survival in the patients receiving adjuvant therapy compared with those who received surgery alone (52 percent three-year survival versus 41 percent, respectively). Preoperative chemotherapy also may be useful in patients with locally advanced GC, offering a chance for surgery with curative intention in patients with an otherwise fatal long-term prognosis [Wilke et al. 1989]. Newer studies [Abe et al. 1981] suggest that intra operative radiotherapy, which allows for a narrowed