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Fabrication of Gold Nanoparticles for targeted therapy in pancreatic cancer**

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Abstract

The targeted delivery of a drug should result in enhanced therapeutic efficacy with low to minimal side effects. This is a widely accepted concept, but limited in application due to lack of available technologies and process of validation. Biomedical nanotechnology can play an important role in this respect. Biomedical nanotechnology is a burgeoning field with myriads of opportunities and possibilities for advancing medical science and disease treatment. Cancer nanotechnology (1–100 nm size range) is expected to change the very foundations of cancer treatment, diagnosis and detection. Nanomaterials, especially gold nanoparticles (AuNPs) have unique physicochemical properties, such as ultra small size, large surface area to mass ratio, and high surface reactivity, presence of surface plasmon resonance (SPR) bands, biocompatibility and ease of surface functionalization. In this review, we will discuss how the unique physico-chemical properties of gold nanoparticles may be utilized for targeted drug delivery in pancreatic cancer leading to increased efficacy of traditional chemotherapeutics.

Keywords

Colloidal gold nanoparticles; AuNPs; fabrication; Targeted therapy; Drug Delivery; Pancreatic Cancer; EGFR; tyrosine kinase; anti cancer drugs

1. Introduction

Cancer is a major public health problem worldwide. Once considered an incurable disease, but today most patients diagnosed with early stage cancer will survive their illness. However, cancer remains the second leading cause of death in the United States, exceeded only by cardiovascular diseases, and accounts for one in four deaths [1]. Solid tumor can grow in different sites in the human body such as prostate, breast, urinary bladder, colon and rectum, kidney and renal pelvis, lung and bronchus, melanoma of skin, pancreas, thyroid, liver and

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intra-hepatic bile duct, esophagus, oral cavity and pharynx or could be of hematopoietic origin such as leukemia, non-hodgkin lymphoma etc [1]. Among these cancers, adenocarcinoma of the exocrine pancreas is the fourth leading cause of cancer deaths in the United States [2]. The most common type, accounting for ~95% of cancer of the pancreas, is adenocarcinoma [3,4]. According to the American Cancer Society about 37,170 individuals in the United States were diagnosed with this condition and an estimated 33,370 deaths were expected to occur in 2007 [5]. Early diagnosis of tumors in the body or tail of the pancreas is difficult because cancer of pancreas often develops without early symptoms [4,5]. Due to its late presentation only 9–15% of patients are suitable for surgical intervention [5–15]. For all stages combined, the 1- and 5-year relative survival rate is 26% and 5%, respectively. The 5-year survival is only 5%, even for those patients diagnosed with local disease. Therefore, new treatment strategies are urgently required to combat this deadly disease.

2. Types of pancreatic cancer

Pancreatic cancer can be divided into two major classes according to the types of cells involved. These are (i) adenocarcinoma- is a cancer that originates in the glandular tissue or pancreatic ducts. Cells that line the ducts of the pancreas help produce digestive juices. The majority of pancreatic cancers are adenocarcinomas. Sometimes these cancers are described as exocrine tumors, and (ii) Endocrine pancreatic cancer- is a disease in which cancerous cells originate within the tissues of the pancreas that produce hormones. Endocrine cancers of the pancreas are rare [9].

3. Cancer nanotechnology: Definition and application

Nanotechnology is a multidisciplinary field that involves the design and engineering of functional systems at the molecular scale (1–100 nm or smaller). “Nano” refers to the scale of objects measured in nanometers (nm) i.e. one nanometer (nm) is one billionth, or 10^{-9}, of a meter. As the dimensions of nanoparticles are similar to biomolecules [such as proteins (1–20 nm), DNA (~diameter 2 nm), virus (~20 nm), cell surface receptors (~10 nm), hemoglobin (~5nm), cell membrane (~6–10 nm)], therefore scientists with diverse interests and backgrounds have converged in their interest to work with and understand properties of materials on a nanoscale and apply them in medicine [14,15].

Cancer nanotechnology is the medical application of nanotechnology that will hopefully lead to useful research tools, advanced drug delivery systems, and new ways to diagnose and treat cancer disease or repair damaged tissues and cells [16]. Cancer nanotechnology is used to characterize the interaction of nanoscale devices with cellular and molecular components specifically related to cancer diagnosis and therapy. Due to their very small size, the surface modified nanoparticles conjugated with therapeutic drugs can penetrate the tumors with a high degree of specificity [14,17]. The National Cancer Institute (NCI) has also recognized that nanotechnology offers the unprecedented and paradigm-changing opportunity to study the normal and cancer cells in real time, at the molecular scale, and during the earliest stages of the cancer progression [18]. To develop cancer nanotechnology, NCI has planned six major challenge areas of emphasis which include: (i) prevention and control of cancer, (ii) early detection and proteomics, (iii) imaging diagnostics, (iv) multifunctional therapeutics, (v) quality of life enhancement in cancer care, and (vi) interdisciplinary training. [18,19].

In the past few years, the applications of nanotechnology have been realized in clinical laboratory analysis, imaging and therapeutics. In cancer therapy, targeted delivery in a localized way is one of the key challenges. Nanotechnology has the potential to play a significant role to achieve such a goal. In cancer therapeutics, nanoparticle-mediated targeted delivery of drugs might significantly reduce the dosage of the drugs with better specificity, low toxicities, and better bioavailability.

4. Importance of nanotechnology in cancer

Since nanoparticles are hundred to thousand times smaller than a human cell (described above), therefore nanoscale devices (50 nm or less) can enter cells and the organelles easily and interact with DNA, proteins, enzymes and cell receptors extracellularly and intracellularly. Again, smaller nanoparticles (≤20 nm) can move out of blood vessels and circulate throughout the body. Since biological processes, including events that lead to cancer, occur at the nanoscale and inside the cells, nanotechnology offers tools that may be able to detect disease in a very small volume of cells or tissue. In general, nanotechnology may offer a faster and more efficient means for scientists to do much of what they do now [18].

5. Signaling pathways in human pancreatic cancers

It is essential to understand the molecular pathogenesis of pancreatic cancer to help to identify suitable targets for chemoprevention. Pancreatic cancer occurs due to a series of genetic mutations which allow a cell to become malignant and activates various intracellular signaling pathways involved in malignant cell growth in an uncontrolled manner [20]. A brief description of signaling pathways related to pancreatic cancer are described below: (i) Mitogen-activated protein kinases (MAPK) belong to kinase family that phosphorylates certain serine or threonine residues in their substrate. Abnormal activity of this pathway can result in malignant cell growth. (ii) Phosphoinositide 3-kinases (PI 3-kinases or PI3Ks) are a family of related enzymes that phosphorylate the 3-position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). Their activity contributes significantly to cellular transformation and the development of cancer. (iii) STAT stands for "Signal Transducer and Activator of Transcription". STATs regulate many aspects of cell growth, survival and differentiation. There are several STAT proteins, of which STAT3 is of particular importance as it up-regulates VEGF, an important growth factor for angiogenesis. Bartsch et al., demonstrated that pancreatic cancers contain an average of 63 genetic alterations, the majority of which are point mutations. These alterations define a core set of 12 cellular signaling pathways and processes that are each genetically altered in 67 to 100% of the tumors. The genetic basis of familial pancreatic cancer (FPC) is unknown. Several genetic alterations have been identified in these lethal cancers, including those in the CDKN2A, SMAD4, and TP53 tumor suppressor genes and in the KRAS oncogene [21–25]. The discovery of these genes, have provided significant insights into the natural history of the disease and to develop improved diagnostic and therapeutic agents. The precise relationship between the CDKN2A gene and pancreatic cancer remains unknown. The CDKNA gene is localized at chromosome 9p21 and encodes the cyclin-dependent kinase inhibitor p16INK4a (MTS1) and the p53 activator p14ARF [26]. Defects in CDKN2a are involved in tumor formation in a wide range of tissues [27]. Inherited mutations in CDKN2A have been found to be associated with other, non-melanoma cancers including pancreatic cancer and neural system tumors. [28].

SMAD4 (also termed DPC4) is a tumor-suppressor gene for pancreatic cancer located on chromosome 18q that mediates the downstream effects of the TGF-β superfamily, resulting in growth inhibition[29–31]. SMAD4 binds to other SMAD proteins forming a complex, which interacts with DNA binding proteins leading to the regulation of transcription and ultimately decreased cellular proliferation. Thus, the loss of SMAD4 expression in pancreatic ductal adenocarcinoma leads to upregulation of cell cycle proteins and hence increases cellular proliferation [32]. The gene (TP53) encodes the protein p53 (also known as protein 53 or tumor protein 53), where it regulates the cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism and thus functions as a tumor suppressor that is involved in preventing cancer [33,34]. p53 protein is expressed at low level in the normal cells and at high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy [35].

Mutated K-Ras gene is associated with 90 percent of pancreatic cancers, as well as highly expressed in other cancers such as non-small cell lung cancer and colorectal cancer. The Ras pathway inhibitors are a growing class of cancer drugs [36]. Ji et al., reported that oncogenic KRAS activates hedgehog (Hh) signaling in pancreatic ductal adenocarcinoma (PDA) cells, utilizing a downstream effectors pathway mediated by RAF/MEK/MAPK but not phosphatidylinositol 3-kinase (PI3K)/AKT [37]. Recently, the hedgehog (Hh) signaling pathway has been implicated in the progression and maintenance of PDA [38]. They suggested that KRAS plays an essential role in the initiation, development, and maintenance of PDA.

5.1. Role of tyrosine kinases in pancreatic cancer

Tyrosine kinases (TKs) play a pivotal role in intercellular signal transduction and regulate crucial cellular processes of tumor cells such as adhesion, proliferation, cell cycle, motility, migration, invasion, differentiation, metabolism, survival, angiogenesis and apoptosis [39–40]. A tyrosine kinase is an enzyme that can transfer a γ-phosphate group from adenosine triphosphate (ATP) (Fig.1) to the hydroxyl group of tyrosine (Fig.1) residues on signal transduction molecules (proteins) [41]. Tyrosine kinases are a subgroup of the larger class of protein kinases. Phosphorylation of proteins (signal transduction molecules) by kinases is an important mechanism in signal transduction for regulation of enzyme activity and it is a major activating event that leads to dramatic changes in tumor growth [42]. Approximately 2000 kinases are known and more than 90 Tyrosine Kinases (TKs) have been found in the human genome and they are mainly classified into two groups: (i) receptor TKs (RTKs) and (ii) non-receptor, cytoplasmic TKs (CTKs) [43–44].

RTKs possess (a) an extracellular ligand-binding domain, which is able to bind a specific ligand, (b) a transmembrane domain, and (c) an intracellular catalytic domain, which is able to bind and phosphorylate select substrates. RTKs are activated by ligand binding to their extracellular domain. Ligands are extracellular signal molecules (e.g. EGF, PDGF etc) that induce receptor dimerization (except Insulin receptor). Different ligands employ different strategies by which they achieve the stable dimeric conformation [45]. Binding of a ligand to the extracellular region causes a series of structural rearrangements in the RTK that lead to its enzymatic activation. Particularly, movement of some parts of the kinase domain provides free access to ATP and the substrate to the active site. This triggers a cascade of events through phosphorylation of intracellular proteins that ultimately transduce the extracellular signal to the nucleus, causing changes in gene expression. Many RTKs are involved in oncogenesis, either by gene mutation, or chromosome translocation [46] or simply by over-expression. RTKs are specifically activated by several growth factors, which include the epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), FGF, and many others [44,47–48].

The non-receptor or cellular tyrosine kinase (CTKs) are located in the cytoplasm, nucleus or are anchored in the inner leaflet of the plasma membrane [43]. They lack a transmembrane segment and generally function downstream of the receptor TKs. They are grouped into eight families – SRC, JAK, ABL, FAK, FPS, CSK, SYK and BTK – each family consisting of several members. Of those CTKs whose functions are known, many – such as SRC – are involved in cell growth [43]. Several TKs - such as EGFR (ErbB-1), HER-2/neu (ErbB-2), VEGFR-2, PDGFR-, c-KIT, FGFR-1, CSF1R, SRC and others - are known to be overexpressed or constitutively activated in pancreatic cancer. Hence, blocking receptor tyrosine kinases (RTKs) and non-receptor, cytoplasmic tyrosine kinases (CTKs) represents a rational approach to treat pancreatic cancer [39].

5.1.1. Tyrosine kinase inhibitors—Therapeutic strategies for targeting RTKs include blocking extracellular receptor domains on tumor cells (antibody type inhibitors), blocking

intracellular kinase–substrate interaction or inhibiting the enzyme's ATP binding site (small-molecule type inhibitors) [39]. Tyrosine kinase inhibitors (TKIs) hold great promise as a therapeutic strategy for the treatment of pancreatic cancer with improved potency, specificity, and efficacy [49]. Indeed, half of 65 kinase inhibitors are currently in clinical trials for targeting RTKs. In particular, cetuximab, the monoclonal antibodies against EGFR-1 (ErbB-1) and erlotinib (a tyrosine kinase inhibitor), showed promising activity in Phase II and Phase III trials and their combination with gemcitabine resulted in synergistic anti-tumor activity. Combinations comprising gemcitabine and tyrosine kinase inhibitors are widely used for the treatment of pancreatic cancer [50].

5.2. Role of epidermal growth factor receptor (EGFR) and cetuximab (C225) in Pancreatic cancer

Epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a 170-kD transmembrane glycoprotein composed of an extracellular ligand-binding domain, a transmembrane region, and a cytoplasmic protein kinase domain involved in signaling pathways essential for cell division and tumor growth [51–53]. EGFR is overexpressed in many types of epithelial tumors, and this typically correlates with aggressive tumor growth [54–57]. EGFR is overexpressed on 22%–60% of human pancreatic carcinomas [53,58–63]. It is also overexpressed in a variety of other cancers such as head, neck, renal, breast, colorectal, prostate, etc. [64,65]. Mutations that lead to EGFR overexpression or overactivity have been associated with a number of cancers [66]. The identification of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR, including gefitinib [67] and erlotinib for lung cancer, and cetuximab for pancreatic and colon cancer [6,68–72]. Targeting of EGFR with monoclonal antibodies has become possible with the recent introduction of chimeric and humanized antibodies. Many therapeutic approaches are aimed at the EGFR. Cetuximab (C225), IgG1 type, anti-EGFR antibody, is one of the examples of monoclonal antibody inhibitors, that has been widely used as targeting agent [73,6,53]. C225 has been approved by FDA (Food and Drug Administration) for the treatment of a variety of EGFR-positive cancers[74–80]. Cetuximab (IMC-C225), a chimeric antibody against EGFR, has shown preclinical activity in a variety of tumor models [63,54,61]. Cetuximab is a monoclonal antibody to epidermal growth factor receptor (EGFR) that blocks the interaction of epidermal growth factor (EGF). Binding of C225 to EGFR leads to receptor internalization and degradation without phosphorylation. When EGFR is stimulated, a series of chemical reactions starts that results in a tumor being "told" to grow. Cetuximab helps stop these reactions by blocking EGFR and consequently stop tumors from growing.

5.2.1. EGFR- receptor targeting in pancreatic cancer—The epidermal growth factor receptor family consists of four tyrosine kinase receptors including ErbB-1 (EGFR) and ErbB-2 receptor (HER-2/neu), ErbB-3 (HER-3) and ErbB-4 (HER-4) [81]. ErbB-1 and ErbB-2 receptor expression and over-expression has been observed in various solid malignancies and has been most extensively studied in human breast cancer [82]. As we have already described that blocking receptor tyrosine kinases (RTKs) should result in suppression of tumor growth, hence it represents a rational approach to treat pancreatic cancer [39].

Two classes of EGF-receptor inhibitors are available: monoclonal antibodies (MABs) [Cetuximab (Erbitux, IMC-C225), Panitumumab (ABX-EGF), Matuzumab (EMD72000), Trastuzumab (Herceptin)] that inhibit ligand binding to EGFRs and small-molecule tyrosine kinase inhibitors (TKIs) [Gefitinib (Iressa, ZD1839), Erlotinib (Tarceva, OSI 774)] that inhibit the tyrosine kinase activity of EGFRs by interfering with ATP binding. Most of the patients with pancreatic cancer overexpress ErbB-1, therefore various ErbB1-MABs and ErbB1-TKIs are under active investigation.

5.2.1.1. Antibody type inhibitors: Cetuximab (Erbitux, IMC-C225): Cetuximab (C225), IgG1 type, anti-EGFR antibody, is one of the examples of monoclonal antibody inhibitors, has been widely used as a targeting agent [73,6,53]. C225 has been approved by FDA (Food and Drug Administration) for the treatment of variety of EGFR-positive cancers [74–80].Cetuximab (IMC-C225), a chimeric antibody against EGFR, has shown preclinical activity in a variety of tumor models [63,54,61]. Cetuximab is a drug that blocks epidermal growth factor receptor (EGFR) that is overexpressed in certain types of cancer, especially pancreatic cancer. Cetuximab acts as a competitive inhibitor to epidermal growth factor (EGF). Binding of cetuximab to the EGFR blocks phosphorylation and activation of receptor-associated tyrosine kinases. This leads to inhibition of cell growth, induction of apoptosis, and decreased production of autocrine growth factors. Cetuximab (C225) is in clinical trials, the main toxicity related to cetuximab has been skin rash and occasional allergic reactions. It exhibited low immunogenicity as less than 4 % of patient developed antichimeric antibodies in phase I clinical trials. Cetuximab costs up to $30,000 for eight weeks of treatment per patient [83]. Cetuximab may cause other side effects including (i) swelling of the hands, feet, ankles, or lower legs, (ii) fast heartbeat, (iii) coughing up blood or dry cough, (iv) shortness of breath or unusual tiredness during exercise, (iv) fainting, (v) decreased urination, (vi) muscle cramps, (vii) shaking of the hands that you cannot control, (viii) sudden tightening of the hands or feet, (ix) twitching of the body that you cannot control, (x) sore throat, fever, chills, and other signs of infection, (xi) diarrhea, (xii) confusion, (xiii) itching, and (xiv) red, swollen, or infected skin etc. [84].

5.2.1.2. Vectibix (Panitumumab: ABX-EGF): Panitumumab (ABX-EGF; Abgenix) was the first fully humanized IgG2 monoclonal antibody (MAb) specific for the ErbB-1 receptor. Panitumumab belongs to a subgroup of monoclonal antibodies, known as epidermal growth factor receptor (EGFR) inhibitors. Panitumumab binds EGF receptor, and prevents it from being activated. This stops the internal cellular signals, and inhibits the growth of cancer cells that have the EGFR on their surface. Panitumumab (Vectibix, Amgen, Inc) was approved by the FDA on September 27, 2006, for the treatment of patients with EGFR-expressing, metastatic colorectal carcinoma. Yang et al., demonstrated that ABX-EGF binds EGFR with high affinity (5×10^{-11}M), blocks the binding of both EGF. They observed a potential anti-tumor activity of ABX-EGF, a human IgG2 monoclonal antibody (mAb) specific to human EGFR, to a variety of human tumor xenografts that express ErbB-1, including pancreatic carcinoma (BxPC-3) [85].

Recently, Amado et al., have reported that the efficacy of Vectibix (panitumumab) for treating metastatic colorectal cancer is limited to patients with tumors lacking KRAS mutations [86]. Conjugated mAbs can increase the specificity of chemo- or radiation therapy and improve the efficacy of immunotherapy, but have some drawbacks; they are more difficult to prepare and may have greater safety issues compared with their naked counterparts. Despite this, immunoconjugates of various kinds constituted 44% of the total anticancer mAbs in clinical study to date [87].

5.2.1.3. Matuzumab (EMD72000): Matuzumab (formerly EMD 72000) is a humanized monoclonal antibody that binds to ErbB-1 (EGFR) with high affinity and specificity, competitively blocking natural ligand binding and blocking receptor-mediated downstream signaling, resulting in impaired tumor cell proliferation, used for the treatment of cancer. Kleespies et al. demonstrated that matuzumab significantly blocks ligand-dependent ErbB-1 phosphorylation and constrains receptor-mediated downstream signaling in human pancreatic cancer cells [88]. Significant antiproliferative, antiangiogenic, antimetastatic, and proapoptotic effects were observed using Matuzumab in pancreatic cancer models. They also observed that in combination with gemcitabine, matuzumab was superior to standard gemcitabine therapy regarding long-lasting antitumor effects and antimetastatic activity [88]. Matuzumab is currently under clinical investigation and has not been approved for use in the US, Europe,

Canada, or elsewhere. It is currently in phase II clinical trials for the treatment of colorectal, lung and stomach cancer. Preliminary results of the colorectal cancer, observed by Merck Serono were less than promising, and it indicates that further trials for treating this type of cancer may be abandoned. [88].

5.2.1.4. Trastuzumab (Herceptin): Trastuzumab (commonly known under the trade name Herceptin) is a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor. The FDA granted approval to trastuzumab (Herceptin®, made by Genentech, Inc.) on November 16, 2006. Trastuzumab after adjuvant chemotherapy significantly improves disease-free survival among women with HER2-positive breast cancer after one of year treatment [89–92]. Though trastuzumab has a "major impact on the treatment of HER2-positive metastatic breast cancer, it has significant complications including cardiac dysfunction (reduced heart function) in 2–7% of cases [93]. As a result, echocardiography is commonly undertaken during the trastuzumab treatment period. Approximately 10% of patients are unable to tolerate this drug because of pre-existing heart problems. Some patients have had serious infusion reactions and lung problems; fatal infusion reactions have been reported. For a pregnant woman, herceptin can cause low amniotic fluid levels and harm to the fetus. Fever, nausea, vomiting, infusion reactions, diarrhea, infections, increased cough, headache, fatigue, shortness of breath, rash, low white and red blood cells, and muscle pain are the most common side effects associated with herceptin [94].

5.2.1.5. Tyrosine kinase inhibitors: Gefitinib (marketed as Iressa): Gefitinib (marketed as Iressa) (Fig.1) is a drug that is used to treat several types of cancers including lung cancer and pancreatic cancer. FDA approved Gefitinib on May of 2003. Although Iressa is approved by FDA, it states that the medicine should be used only in cancer patients who have already taken the medicine and whose doctor believes it is helping them. Gefitinib is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain. EGFR is overexpressed in the cells of certain types of human carcinomas - for example in lung, pancreatic and breast cancers.

Tyrosine kinases (TKs) play a pivotal role in intercellular signal transduction and regulate crucial processes of tumor cells such as proliferation, migration, survival and angiogenesis. Gefitinib attaches to EGFRs and inhibits EGFRs by binding to the adenosine triphosphate (ATP)-binding site of the enzyme and thereby blocks the attachment of EGF and the activation of tyrosine kinase [95–96]. Thus the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is inhibited, and malignancy is inhibited. This mechanism for stopping cancer cells from growing and multiplying is very different from the mechanisms of chemotherapy and hormonal therapy [97–98]. Li et al., demonstrate that gefitinib inhibits pancreatic cancer cell growth through EGFR-dependent pathways [99]. A major drawback of studies of EGFR-TKIs in breast cancer is the absolute lack of criteria to select patients that are likely to respond to these agents. Therefore, identification of such criteria is mandatory to improve the efficacy of this approach[100]. Diarrhoea, nausea, vomiting, anorexia, stomatitis, dehydration, skin reactions, paronychia, asymptomatic elevations of liver enzymes, asthenia, conjunctivitis, blepharitis are the most common adverse side effects associated with the use of gefitinib. Apart from that interstitial lung disease, corneal erosion, aberrant eyelash and hair growth are infrequent adverse effects (0.1–1% of patients) [101].

5.2.1.6. Erlotinib (Tarceva, OSI 774): TARCEVA (erlotinib), a kinase inhibitor, is a quinazolinamine with the chemical name N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine. TARCEVA contains erlotinib as the hydrochloride salt that has the following structural formula: (Fig. 1). Erlotinib hydrochloride is a drug that is used to treat several types of cancers including lung cancer, pancreatic cancer etc. Erlotinib attaches to EGFR and thereby blocks the attachment of EGF and the activation of tyrosine kinase. On November 18, 2004,

the FDA approved erlotinib hydrochloride (Tarceva™ tablets, made by OSI Pharmaceuticals Inc.). Moore et al., demonstrated that statistically significant improved survival was observed in advanced pancreatic cancer in randomized phase III trial by adding any agent to gemcitabine [102].

Similar to other therapies, a potential drawback of EGFR-based therapy is the possibility that patients will develop resistance the treatment. For example, some patients who initially responded to gefitinib or erlotinib therapy develop resistance to these therapies after prolonged treatment. However, current treatment techniques do not allow for a rapid and noninvasive determination of whether patients receiving EGFR-based therapy are responding to treatment and when it is appropriate to discontinue therapy if the patient is not responding. As a result, patients with lung cancer may receive an ineffective treatment for extended periods of time. Another major clinical problem is the inability to demonstrate whether the EGFR-targeted inhibitors specifically targeted the lung tumors to produce the desired therapeutic effect [103].

5.2.1.7. EKB-569: This drug is being studied in the treatment of cancer and it belongs to the family of drugs called epidermal growth factor receptor (EGFR) inhibitors. A 3-cyanoquinoline pan-ErbB tyrosine kinase inhibitor with potential antineoplastic activity. EKB-569 covalently binds to epidermal growth factor receptors (EGFR) ErbB-1, -2 and -4 irreversibly, thereby inhibiting receptor phosphorylation and signal transduction and resulting in apoptosis and suppression of proliferation in tumor cells that overexpress EGFR receptors [104]. Hidalgo et al., demonstrated that EKB-569 was generally well tolerated, with an acceptable PK and safety profile, and offers a promising targeted approach to the treatment of solid tumors [105]. Combination therapy of EKB-569 and gemcitabine has generally been well tolerated in advanced pancreatic cancer [106]. However, dose–limiting toxicities were grade three diarrhea and elevated transaminases at doses of 100 mg/day. Pharmacokinetics of EKB-569, safety of EKB-569 and efficacy data has not been well established. Erlichman et al., demonstrated that the EKB-569–related adverse events of any grade that occurred in any cycle in at least 10% of the patients in the continuous-dose group. Diarrhea, rash, and asthenia occurred with the highest incidence [104].

5.2.1.8. PKI-166 (CGP75144): PKI-166 (Novartis), a pyrrolo-pyrimidine derivative, is a selective inhibitor of the tyrosine kinase activity of the EGFR and the erbB2 receptor (HER2/neu). Baker et al., have shown the inhibition of protein tyrosine kinases of EGFR (PKI 166) combined with gemcitabine can significantly reduce the growth and metastatic potential of highly metastatic human pancreatic cancer growing in the pancreas of nude mice [68]. PKI-166 (Novartis) is a selective inhibitor of the tyrosine kinase activity of the EGFR and the erbB2 receptor (HER2/neu). According to phase I and pharmacologic studies of PKI-166 in Netherlands and Belgium, initially PKI-166 was administered on a daily schedule without interruption, but after 4 grade 3 transaminase elevations occurred in the first 2 dose cohorts (50 mg and 100 mg orally), different dosing schedules were initiated. Dose-limiting toxicities included grade 3 transaminase elevation, skin rash, and diarrhea, observed in 3 of 4 evaluable patients at 900 mg [107,108].

5.2.1.9. Tykerb (*Lapatinib ; GW572016*): Lapatinib is a small molecule and a member of the 4-anilinoquinazoline classes of kinase inhibitors (Fig. 1). Lapatinib (INN), FDA approved, is an orally active chemotherapeutic drug used for the treatment solid tumors such a breast cancer [109]. According to Baerman et al., lapatinib inhibits EGFR dependent proliferation and anchorage-independent colony formation in pancreatic cancer cell lines through inhibition of MAPK and Akt pathways [110]. The use of Tykerb can cause some serious side effects which includes uneven heart rate, extreme dizziness or fainting, severe diarrhea; dry cough, feeling short of breath, white patches or sores inside your mouth or on your lips nausea, stomach pain,

low fever, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes).

5.3. Other receptor inhibitors

Several TKs – other than EGFR (ErbB-1) and HER-2/neu (ErbB-2), such as VEGFR-2, PDGFR-, c-KIT, FGFR-1, CSF1R, SRC - are known to be overexpressed or constitutively activated in pancreatic cancer. Hence, blocking receptor tyrosine kinases (RTKs) and non-receptor, cytoplasmic tyrosine kinases (CTKs) represents a rational approach to treat pancreatic cancer. However here we will not discuss in detail about these inhibitors. Briefly, small molecule inhibitors (VEGF-receptor TKIs) like Vatalanib, zactima, and semaxanib have been used for targeting VEGF-receptors in pancreatic cancer. Imatinib, sorafenib, leflunomide are being used to target PDGFR in variety of cancers. Finally, Src kinase inhibitors such as pyrazolopyrimidines, AZM475271, siRNA, AP23846, SKI-606, AZD05230 etc are also being used for the treatment of cancers [39].

6. Standard chemotherapy of pancreatic cancer

Chemotherapy uses drugs to help kill cancer cells. Chemotherapy based on the following drugs has been shown to prolong survival in advanced pancreatic cancer. Readers interest ed in chemotheraputic approaches for pancreatic cancer can refer to the following article [111].

6.1. 5-fluorouracil (5-FU) (Fluoropyrimidines)

5-FU is a pyrimidine analog, which is used as a drug in the treatment of variety cancers (Fig. 1). 5-FU is the most commonly administered treatment for patients with advanced or metastatic pancreatic cancer [39]. 5-Fu is S-phase–specific, fluorinated pyrimidine that is metabolized intracellularly to its active form fluorodeoxyuridine monophosphate (FdUMP) via the *de novo* pyrimidine pathway; and then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell's ability to synthesize DNA [112–116]. In addition to being incorporated in DNA and RNA, the drug has been shown to inhibit the activity of the exosome complex, an exoribonuclease complex essential for cell survival [117]. Before the development of gemcitabine, only 5-FU and mitomycin C demonstrated a beneficial effect in pancreatic cancer [118–120]. 5-FU has some serious side effects including myelosuppression, mucositis, dermatitis, diarrhea and cardiac toxicity. 5-FU also causes both acute central nervous system (CNS) damage and progressively worsening delayed degeneration of the CNS in mice. This latter effect is caused by 5-FU-induced damage to the oligodendrocytes that produce the insulating myelin sheaths[121].

6.2. Gemcitabine

Gemcitabine is a drug that is commonly used for the treatment of several cancers including lung cancer, pancreatic, and bladder and breast cancer. Gemcitabine is a pyrimidine analog or nucleoside analog (Fig. 1) with a wide spectrum of antitumor activity [63]. Gemcitabine currently is used as the drug of choice for treatment of pancreatic cancer [39]. The chemical name of gemcitabine is (2′,2′-difluoro-2′-deoxycytidine or 1-(2-oxo-4-amino-1,2-dihydropyrimidin-1-yl)-2-deoxy-2,2-difluororibose hydrochloride. It is metabolized intracellulary by nucleoside kinases to the active species gemcitabine-diphosphate (dFdCDP) and gemcitabine-triphosphate (dFdCTP). Incorporation of dFdCTP into DNA is responsible for the cytotoxic effects of gemcitabine, via inhibition of DNA synthesis, DNA repair and ultimately via induction of apoptosis. In 1998 the FDA approved Gem for use in palliative treatment of patients with pancreatic carcinoma. It shows modest survival benefit compared to 5-fluorouracil (5-FU), the commonly administered treatment for patients with advanced or metastatic pancreatic cancer [122–124]. Moreover, Gem is also used in the treatment of other malignancies such as head and neck, lung, breast and ovarian cancers [125].

A number of other single agents have been evaluated without significant results in patients with advanced pancreatic cancer, including raltitrexed [126], irinotecan [127], topotecan [128], iproplatin [129], trimetrexate [130], edatrexate [131], farazarabine [132], diaziquone (AZQ) [133], mitoguazone (MGBG)[134], and amonafide [135]. Ifosfamide [136,137] showed promise in early trials (response rates, 22% and 17%); however, its efficacy was not substantiated by an MDACC study in which the overall response rate was only 7% [138]. Similarly, an early study of docetaxel [139] demonstrated positive results (response rate, 29%) that were not confirmed in a subsequent phase II trial at MDACC and Sloan-Kettering (response rate, 17% [140]. Gemcitabine is the current standard chemotherapy for advanced pancreatic cancer, but is still far from optimal and novel therapeutic strategies are urgently needed [39]. It possesses important drawbacks like a poor biological half-life and the induction of resistance [141]. Unfortunately, gemcitabine possesses a rapid body clearance that limits its efficacy, a drawback due to kidney excretion and metabolism by the plasmatic enzyme cytidine-deaminase, which yields the inactive metabolite 2′,2′-difluorodeoxyuridine (dFdU) [142]. Thus, a frequent administration schedule at high drug doses is required and this leads to significant side effects [142,143].

6.3. Combination Therapy (cetuximab and gemcitabine)

Combination chemotherapy has been demonstrated to be better than single agents for many solid tumors. The combination of cetuximab and gemcitabine was at least additive in preclinical models [73,63,144]. Treatment-related toxicities were mild to moderate that included skin rash, fatigue, and fever. These exciting results prompted investigators in cancer research to design a better and alternative targeted drug delivery system (DDS) including gemcitabine with or without cetuximab. Therefore this strategy could be used as a generalized approach for the treatment of a variety of cancers including pancreatic cancer. A preliminary report indicated that the combination of gemcitabine with 5-FU was well tolerated and showed promising antitumor activity against pancreatic cancer [145].

The HER2/neu oncogene is overexpressed in (up to 70%) human pancreatic cancer specimens when compared to normal pancreatic tissue. This cell surface receptor (HER2) can be targeted specifically by the neutralizing antibody Herceptin. Buchler et al., has investigated the therapeutic efficacy of Herceptin in combination with gemcitabine and docetaxel and observed that combination therapy resulted in a dramatic improvement of animals bearing human pancreatic cancer xenografts [146]. In 2007, Miyake et al., demonstrated the antitumor and antiangiogenic activities of human natural interferon-alpha (IFN-α) alone or in combination with S-1 against human pancreatic cancer cells. Their data suggested that administration of IFN-α in combination with S-1 may provide a novel and effective approach to the treatment of human pancreatic cancer [147]. In 2008, Lee et al., demonstrated that combination of gemcitabine and apigenin augmented tumor growth inhibition through the down-regulation of NF-kappa B activity with the suppression of Akt in tumor tissue *in vivo*. The combination of gemcitabine and apigenin enhanced anti-tumor efficacy through Akt and NF-kappa B activity suppression and apoptosis induction [148].

7. Tumor markers in pancreatic cancer

The two markers that are commonly used in the management of pancreatic cancer are carcinoembrionic antigen (CEA) and carbohydrate antigen (CA 19-9). [149].

7.1. Carbohydrate antigen (CA) 19-9 [CA-19-9]: (useful tumor marker for diagnosis of exocrine pancreatic carcinoma)

CA 19-9 [carbohydrate antigen 19-9 o sialylated Lewis (a) antigen] is a tumor-associated antigen, or tumor marker, that is frequently elevated in the serum or plasma of patients that

have been diagnosed with cancer of the pancreato-biliary system (i.e. pancreas, gallbladder, biliary tract). CA 19-9 has been investigated as a prognostic and screening tool in pancreatic cancer and is thought to be the most useful serum marker for this disease [150]. It was discovered in patients with colon cancer and pancreatic cancer in 1981 [151]. In addition, elevated CA 19-9 values have been observed in other malignancies such as lung cancer, colonic, ovarian carcinoma, hepatocellular cancer, other gastrointestinal cancers, and in some nonmalignant disorders. The concentration of the serum tumor marker carbohydrate antigen (CA) 19-9 is increased in more than 80% patients with advanced pancreatic carcinoma, and is routinely used to monitor the course of disease, both on and off treatment [152–156]. To determine the level of CA 19-9 in the blood, a blood sample is taken from the patient and then sent to a laboratory for testing. [156] The amount of antigen present in the blood sample is measured by using a monoclonal antibody known to specifically bind to the CA 19-9 antigen. This test has been used to monitor disease status in those patients having confirmed pancreatic cancer who have levels of serum or plasma CA 19-9 above the cutoff, at the time of diagnosis [157]. The concentration of CA 19-9 higher than 37 U/ml is considered as abnormal. The higher the number, the more advanced the disease. The measurement of this concentration is convenient, cheaper, and easier than the measurement of target lesions on standard imaging, which are usually difficult to assess in this disease. Therefore, the use of CA 19-9 response as an endpoint allows the inclusion of patients with unmeasurable disease into clinical trials. Finally, CA 19-9 has been used in the diagnosis of pancreatic cancer but is also a marker of pancreatic tissue damage which might be caused by diabetes [155].

7.2. Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) is a glycoprotein with a substantial carbohydrate component, secreted by normal mucus-secreting epithelial cells and it is involved in cell adhesion. The word "carcinoembryonic" represents the fact that CEA is produced by some cancers ("carcino-") and by the developing fetus ("-embryonic"). CEA is a type of protein that can be found in many different cells of the body, but is typically associated with certain tumors and the developing fetus [158]. CEA was first identified in 1965 by Phil Gold and Samuel O. Freedman in human colon cancer tissue extracts [159]. It was observed that serum from individuals with colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, lung carcinoma and breast carcinoma, as well as individuals with medullary thyroid carcinoma, had higher levels of CEA than healthy individuals [160]. The normal range for CEA in an adult non-smoker is <2.5 ng/ml and for a smoker <5.0 ng/ml. CEA is most frequently tested in blood. It can also be tested in body fluids and in biopsy tissue. A rising CEA level indicates progression or recurrence of the cancer. In addition, levels >20 ng/ml before therapy are associated with cancer which has already spread (metastatic disease) [158]. The CEA test is ordered for patients with known cancers including cancer of the colon, rectum, stomach (gastric cancer), esophagus, liver, or pancreas. It is also used with cancers of the breast, lung, or prostate [161]. The carcinoembryonic antigen (CEA) test is used to (i) determine how widespread cancer is for some types of the disease, especially colon and pancreatic cancer, (ii) check the success of treatment for colon and pancreatic cancer, (iii) check to see if cancer has relapsed after treatment[162].

8. Targeted drug delivery to pancreatic cancer using nanotechnology

Combination chemotherapy has been demonstrated to be better than single agents for many solid tumors. The combination of cetuximab and gemcitabine has been used in preclinical models [73,63,144]. Again, both cetuximab and gemcitabine have been approved by FDA. Treatment-related toxicities were mild to moderate that included skin rash, fatigue, and fever. These exciting results using a combination of cetuximab and gemcitabine prompted investigators involved in cancer nanotechnology research to design better and alternative

targeted drug delivery system (DDS) for the treatment of variety of cancer, especially pancreatic cancer.

In this context, our group has developed a nanoparticle based targeted drug delivery system (DDS), which contains cetuximab (C225) anti-epidermal growth factor receptor (EGFR) antibody as targeting agent, gemcitabine as anticancer drug, and gold nanoparticles as delivery vehicle [6]. We have demonstrated that administration of this targeted delivery system resulted in significant inhibition of pancreatic tumor cell proliferation *in vitro* and orthotopic pancreatic tumor growth *in vivo* [6]. Therefore, this strategy could be used as a generalized approach for the treatment of a variety of cancers including pancreatic cancer in the near future.

9. Medicinal use of gold nanoparticles

Gold and its compounds have long been used as medicinal agents throughout the history of civilization and described in literature [14,163–169]. Medicinal use of gold has been described briefly below: The earliest records of the use of gold for medicinal and healing purposes come from Alexandria, Egypt. Over 5,000 years ago, the Egyptians ingested gold for mental, bodily and spiritual purification [164]. The ancient Greeks used finely ground gold to color glass, which paradoxically turned it a rich ruby red [170]. The earliest medical use of gold can be traced back to the Chinese in 2500 BC [165,166]. They were the first to prepare and use red colloidal gold as the, “drug of longevity.” Red colloidal gold is still in use today in India in the form of Ayurvedic medicine for rejuvenation and revitalization during old age under the name of Swarna Bhasma (“Swarna” meaning gold, “Bhasma” meaning ash) [171,172]. A gold piece was implanted under the skin near an inflamed joint, such as a knee or elbow by surgeons in the 1900s. As a result, the pain would often subside or cease altogether [164].

In the nineteenth century, colloidal gold was commonly used to cure alcoholism in the US, and until today it is used to reduce dependency on alcohol, nicotine, caffeine, and carbohydrates. Since 1927, gold has been used to treat arthritis. Today gold, especially gold nanoparticles (AuNPs) has become an important biomedical tool for scientists in cancer research due to several advantages of AuNPs (discussed later). Recently several groups including ours have demonstrated that AuNPs possess an enormous potential to improve the efficacy of cancer treatment [14,6,173–181,163,182–185].

10. Importance of gold Nanoparticles

There are several reasons for the use of AuNPs in nanotechnology as well as in cancer nanotechnology. (i) First of all, gold compounds have long been used in medicine throughout the history of civilization [14,163,165–172]. (ii) It is easy to synthesize AuNPs by several simple, economically cheap, safe and reliable methods such as wet chemical, physical and biological; (iii) It can be synthesized from size 2–500 nm by changing the reaction parameters; (iv) it can be easily synthesized with different shapes (spheres, rods, tubes, wires, ribbons, plate, cubic, hexagonal, triangular) using templates and changing reaction conditions; (v) due to presence of negative charge on surface of AuNPs, they are highly reactive, which helps to modify the surface of AuNPs using several biomolecules. Due to strong interaction between the gold surface and thiol/amine containing molecules (organic molecules, DNA, protein, enzyme etc.) surface of AuNPs can be easily modified; [177] (vi) AuNPs can be easily characterized due to presence of the characteristic surface plasmon resonance (SPR) bands [186]; due to presence of unique optical as well as electronic behavior, these gold particles can be used in biosensors; (vii) Finally, it is well established that AuNPs are biocompatible and non-toxic [187–189,181]. Recently several groups including our groups have demonstrated that AuNPs possess enormous potential to improve the efficacy of cancer treatment [14,6, 173–181,163,182–185].

11. Toxicity, biocompatibility of AuNPs and its diagnostic application

Noncytotoxic, nonimmunogenic, and biocompatible properties of gold nanoparticles are important issues for the potential application in nanoimmunology, nanomedicine, and nanobiotechnology. In this context, several groups have demonstrated the noncytotoxic behavior of gold nanoparticles (AuNPs) [190,191,187,192,193]. For example, Shukla et al. have addressed the issue of cytotoxicity and immunogenic effects of gold nanoparticles on RAW264.7 macrophage cells, one of the principal immune effector cells that play essential roles as secretory, phagocytic, and antigen-presenting cells in the immune system [190]. Using different physicochemical techniques they have correlated the cytotoxicity of gold nanoparticles. They concluded that Au(0) nanoparticles were not cytotoxic, reduced the production of reactive oxygen and nitrite species, and did not elicit secretion of proinflammatory cytokines TNF-α and IL1-β, making them suitable candidates for nanomedicine. Similarly, Pan et al. demonstrated that the cytotoxicity of TPPMS/TPPTS-modified gold nanoparticles depended primarily on their size and not on ligand chemistry [194]. They observed that gold nanoparticles of 1–2 nm in size were highly toxic and both smaller gold compounds (Tauredon) and larger 15-nm gold colloids were comparatively nontoxic. In another study, Fan et al. reported the effects on biocompatibility of water-soluble AuNPs with different sizes and concentrations to human bone marrow mesenchymal stem cells (hBMSCs) and human hepatoma carcinoma cells (HuH-7) [191]. They observed more than 80% cell survival when both cells were incubated with 71.1 μg/mL of 15 and 30 nm AuNPs. Cho et al. found that the 13 nm sized PEG-coated gold nanoparticles were seen to induce acute inflammation and apoptosis in the liver [192]. These toxicity and kinetics findings of PEG-coated gold nanoparticles may have important clinical implications regarding the safety issue as PEG-coated gold nanoparticles are widely used in biomedical applications.

Gold nanoparticles (AuNPs) have exceptional stability against oxidation and therefore will play a significant role in the advancement of clinically useful diagnostic [187,195,193,194] and therapeutic nanomedicines. Kattumuri et al have demonstrated that X-ray CT contrast measurements of gum-arabic matrix vectors using AuNPs (GA-AuNPs) would be useful for potential diagnostic (molecular imaging) and therapeutic applications in nanomedicine. [195] In this context, Eck et al demonstrated the optical detection of antibody-conjugated gold nanoparticles (15 nm spherical) bound to surgically resected human pancreatic cancer tissue. [196] This group has fabricated gold nanoparticle–antibody bioconjugates which is highly stable dispersions and exhibit long-term resistance to agglomeration, observed by dynamic light scattering, size exclusion chromatography, and transmission electron microscopy etc. The bioconjugated nanoparticles were used to label tumor stroma in approximately 5 μm thick sections of resected human pancreatic adenocarcinoma. The tissue samples were imaged by darkfield microscopy near the nanoparticle resonance scattering maximum (560 nm). The images displayed pronounced tissue features and suggest that this novel labeling method could provide for facile identification of cancer tissue.

12. Targeted therapy using gold nanoparticles in pancreatic cancer

Recently several groups including our groups have demonstrated the synthesis of AuNPs [186,14,197–201]. [202–207] physical methods, [208–219] [220–227].and its enormous potential to improve the efficacy of cancer treatment [14,6,173–181,163,182–185]. An ideal therapeutic approach would be to deliver multiple-drugs specifically to the primary tumor, as well as to the site of metastasis and its microenvironment while simultaneously monitoring the prognosis through noninvasive approaches. In cancer therapy, targeted delivery in a localized way is one of the key challenges. Nanotechnology has the potential to play a significant role to achieve such a goal. It is anticipated that nanoparticle-mediated targeted delivery of drugs

might significantly reduce the dosage of the anti-cancer drugs with better specificity, enhanced efficacy and low toxicities.

In this context, it is well established that tyrosine kinase (TKs) - such as EGFR (ErbB-1), is overexpressed in pancreatic cancer. Hence, blocking receptor tyrosine kinases (RTKs) represents a rational approach to treat pancreatic cancer [39]. Therefore, we have developed a gold nanoparticle-based (~5 nm) targeted delivery system (DDS) for *in vitro* and *in vivo* therapeutic application in pancreatic cancer. This DDS was fabricated using gold nanoparticles (AuNPs) as delivery vehicle, gemcitabine as anti cancer drug and cetuximab (C225) anti-epidermal growth factor receptor (EGFR) antibody as targeting agent. There are several reasons for choosing epidermal growth factor receptor (EGFR) as a target in pancreatic cancer. Briefly, EGFR exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGFα). It consists of an extracellular ligand binding domain, a hydrophobic transmembrane domain [75,228,62,54, 61] and an intracellular tyrosine kinase domain. Ligand binding to the EGFR induces receptor homo/heterodimerization, which in turn, leads to intracellular phosphorylation of tyrosine residues. Phosphorylation of EGFR tyrosine kinase activates a complex down stream signaling process the end point of which is proliferation, migration, invasion, and inhibition of apoptosis [54,61]. Mutations affecting EGFR expression or activity could result in cancer. More importantly, it is a FDA approved material for the treatment of several types of cancer.

Similarly, the reasons for choosing gemcitabine as drug are also manifold. It is not only the front line chemotherapy for pancreatic cancer, but also used for the treatment of breast, head and neck as well as ovarian cancer [229–231,215]. We have demonstrated that administration of this targeted delivery system resulted in significant inhibition of pancreatic tumor cell (PANC-1, AsPC-1 and MIA Paca2) proliferation *in vitro* and orthotopic pancreatic tumor growth *in vivo* [6]. This strategy could be used as a generalized approach for the treatment of a variety of cancers characterized by overexpression of EGFR.

12.1. Synthesis and characterization of gold nanoconjugates for the treatment of pancreatic cancer

The AuNPs were synthesized by the reduction of chloroauric acid ($HAuCl_4$) and sodium borohydride ($NaBH_4$) according to our published literature [176–178,6]. The DDS containing gold naoparticles (AuNPs), anti-EGFR antibody (C225) and gemcitabine was fabricated by a two step incubation processes (at pH = 7.8): in the first step AuNPs were incubated with C225 at room temperature (RT) under stirring followed by a second incubation process that involves incubation with gemcitabine for additional 1h under the same condition. The targeted DDS thus formed were physico-chemically characterized by UV-Visible spectroscopy (UV-Vis), transmission electron microscopy (TEM), thermogravimetric analysis (TGA), X-ray photoelectron spectroscopy, radioactivity measurement and HPLC analysis [14].

The exact mechanism of bonding of protein molecules to AuNPs is still poorly understood, however some of the accepted mechanisms are (i) electrostatic interaction, (ii) chemical interactions, (iii) hydrophobic interaction. [232–234]. Stability studies of the nanoconjugates under different environment suggest that the targeted DDS system was fairly stable in cell growth media and in mouse plasma and C225 and Gem are bound to AuNP through pseudo-covalent interaction (Figure 2) [180,186,235–240].

The human EGFR is a transmembrane glycoprotein [54,61,241–243]. It consists of an extracellular ligand binding domain, a hydrophobic transmembrane domain and an intracellular tyrosine kinase domain. Ligand binding to the EGFR induces receptor homo/ heterodimerization, which in turn, leads to intracellular phosphorylation of tyrosine residues. Phosphorylation of EGFR tyrosine kinase activates a complex down stream signaling process

the end point of which is proliferation, migration, invasion, and inhibition of apoptosis. Functional activity of the nanoconjugates in vitro demonstrated that targeted DDS was much more effective to inhibit the proliferation of pancreatic cancer cells than its non-targeted counterpart.

12.2. Selection of a preclinical model for *in vivo* study

The selection of an appropriate model system in which to assess the efficacy of a targeted nanodelivery system in cancer is another very important factor. To validate the efficacy of our nanodelivery system we selected pancreatic cancer as a model as no effective therapy is currently available against pancreatic cancer [244].

As we have discussed already, it is very important to select an appropriate animal model to assess the targeting efficacy of a delivery system. [245] Traditionally therapeutic efficacy is tested in human tumor xenografts implanted subcutaneously (s.c.) in nude mice [246]. This type of model is easy to operate (technically straight forward). But the major limitation of these models is that they do not reproduce the primary site of the common human cancers nor do they represent the common sites of metastasis. On the other hand, the advantage of using orthotopic model (tumors developing in original site) is that they reproduce the primary site of the tumor and closely mimics human metastasis. [247–250] The most obvious limitation of orthotopic model being the technical skill required implanting the tumor cells in the pancreas. Furthermore, the end points to determine the effect of therapy is not straight forward than the normal tumor measurement in s.c. model. Therefore, we believe orthotopic model is a better way of testing in vivo efficacy of a targeted delivery system. Recently, we demonstrated the generation of orthotopic human xenograft model of pancreatic cancer where tumor progression can be monitored non-invasively by bioluminescence from the implanted cells [251]. After orthotopically implanting AsPC-1 in pancreas, luciferase bioluminence in mice was noninvasively imaged using the Xenogen (IVIS 100 imaging system) instrument to check for tumor growth before treatment. Biodistribution studies as determined by inductively coupled plasma analysis demonstrated minimal uptake in vital organs such as liver, kidney whereas significant accumulation of gold was achieved in the tumor (Fig. 3A). Fig. 3.B and 3.C represented the luciferase imaging of the control group (C225 + Gem) and experimental group (Au-C225-Gem), respectively, at the end of the study. Significant tumor growth inhibition was observed when mice were treated with Au-C225-Gem compared with its nontargeted counterpart. These results were further confirmed by measuring the tumor growth after sacrificing the mice at the end of the experiment and assessing the tumor volumes. Au-C225-Gem inhibited tumor growth significantly (~80%) compared with all other nontargeted groups as shown in Fig.3.D (Left).

Inhibition of pancreatic cancer growth with such a low dose of gemcitabine is significant in anticancer therapy where toxicity is one of the major issues. Thus delivery of cytotoxic drug in a targeted fashion is expected not only to increase the efficacy but to reduce the systemic toxic effects because low doses will be required under such delivery option.

13. Radiofrequency ablation (RFA)

Radiofrequency ablation (RFA) is a minimally invasive treatment for cancer that is approved by the FDA. RFA is performed to cure tumors in lung, liver, kidney, bone and rarely in other body organs, yet it suffers from serious limitations [252–256]. The treatment is an alternative when surgery is not likely to be successful or has failed [257,258]. This method has several advantages which include (i) effective treatment for small cancers, (ii) minimally invasive, with no skin incision, (iii) minimal risk to patient, (iv) typically little or no pain, (v) minimal hospital stay, (vi) can be repeated if new cancer appears, (vii) it can be used to treat tumors that are not surgically resectable because of anatomic constraints or inadequate liver reserve,

(viii) reduced morbidity and mortality, and (ix) it is technically easier to perform than surgical resection. However RFA has some limitations which include: (i) RFA is currently an invasive treatment requiring insertion of needle electrodes directly into the tumor(s) to be treated; (ii) incomplete tumor destruction occurs in 5% – 40% of the treated lesions, particularly if lesions are > 4–5 cm in diameter; (iii) the treatment is nonspecific with both malignant and normal tissues around the needle electrode undergoing thermal injury; (iv) complications arise in up to 10% of patients, frequently related to thermal injury to normal tissues; (v) and invasive RFA is limited to treatment of tumors in only a few organ sites (liver, kidney, breast, lung, bone) [184].

In this context, AuNPs are particularly interesting as a therapeutic target for non-invasive RF because a number of gold preparations are already used in clinical practice. After internalization of AuNPs into cells, they serve as target molecules to produce increased intracellular heat when exposed to the external RF field. Recently, Curley and co-workers demonstrated that increased percentage of cell death in the AuNPs-treated cells exposed to an external RF field [184]. It is clear from their investigation that as an intracellular target molecule, AuNPs released substantial heat in the nanoenvironment after exposure to a high-voltage focused RF field. These results demonstrated the increased percentage of cell death in the GNP-treated cells exposed to the external RF field. TEM reveals disruption and destruction of normal intracellular structures and architecture. These results also indicate that AuNPs are suitable targets for RF-induced thermal destruction of cancer cells [184].

14. Photothermal therapy for treatment of cancer

Photothermal Therapy (PTT) is an experimental use of electromagnetic radiation (mostly in the form of infrared; 650–900 nm) [259,260] which has been used to treat various medical conditions, including cancer [261]. Cancer patients without any other alternative for treatment, now have a choice, to treat their cancer using nanophotothermolysis. Unique properties such as absorption and scattering of electromagnetic radiation have been used in photothermal therapy.

Recently, El-Sayed et al., has demonstrated a new way to kill cancer cells more specifically using the unique tunable absorption wavelength at 530 nm (plasmon resonance absorption) and at 650–900 nm (near infrared absorption) of antibody conjugated AuNPs as photothermal agent and published a series of papers in this area of research [262,263,227,264–266]. Based on their previous work [263] that used gold nanoparticles to detect cancer, now the particles are heated using them as thermal agents to destroy malignant cells.

Many cancer cells overexpress the epidermal growth factor receptor (EGFR), along their surface, while healthy cells typically do not express the protein as robustly. Using this concept, the researchers have fabricated a delivery system containing gold nanoparticles (AuNPs) as a delivery vehicle and anti-EGFR as a targeting agent. They have demonstrated that the gold nanoconjugates can specifically target to cancer cells due to presence of EGFR whereas it does not reach normal cells due to lack of EGFR. Therefore accumulation of AuNPs in cancer cells is much higher than that of normal cells. If now both type cells are exposed to continuous visible laser at 514 nm, then more heat will be generated in the cancer cells compared to the normal cells, suggesting that this technique will destroy cancer cells more specifically.

In this study, the researchers incubated two oral squamous carcinoma cell lines (HSC 313 and HOC 3 clone 8) and one benign epithelial cell (HaCaT) line with anti-EFGR conjugated AuNPs and then exposed them to continuous visible argon laser at 514 nm. [262] They observed that malignant cells required less than half the laser energy to be killed than the benign cells.

It is very simple and well established that by changing the shape of AuNPs to gold nanorods with various aspect ratios, one can not only change the absorption and scattering band from visible to the NIR region, but also increase their absorption and scattering cross sections [267,268,218]. The absorption band of core-shell nanoparticles particles were tuned by adjusting the ratio of the thickness of the gold shell to the diameter of the silica core (~120 nm in diameter) and thus enables photothermal therapy in the near infrared (NIR) region (650–900 nm) [259,260]. In principle, the dual imaging/therapy with immunotargeted nanoshells were used to detect and destroy breast carcinoma cells that over express HER2, a clinically relevant cancer biomarker.

Recently, Bhatia and co-workers have demonstrated a computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas [269]. They have demonstrated an integrated approach to improved plasmonic therapy composed of multimodal nanomaterial optimization and computational irradiation protocol development. They synthesized polyethylene glycol (PEG)-protected gold nanorods (NR) that exhibited better spectral bandwidth, and photothermal heat generation per gram of gold than the gold-silica nanoshell. It also demonstrated a long circulation half-life in vivo (t(1/2), approximately 17 hours) than gold nanoshells. Furthermore, it also exhibited approximately 2-fold higher X-ray absorption than a clinical iodine contrast agent. In computationally driven pilot therapeutic studies, they demonstrated that a single i.v. injection of PEG-NRs enabled destruction of all irradiated human xenograft tumors in mice. These studies underline the importance of integrating computational therapy design with nanotherapeutic development for ultraselective tumor ablation. These strategies may be used a generalized approach in future to treat solid tumors.

15. Future direction

Nanotechnology, the creation of new objects in nanoscale dimensions, is a cutting edge technology having important applications in modern biomedical research for cancer detection, diagnosis, and therapy. Looking into the future, there are a number of research themes or directions that are particularly promising but require concerted effort for success. The first direction is the design and development of nanoparticles with monofunctions, dual functions, three functions, or multiple functions. For example, DDS can be varied with one drug or combination of two drugs or multiple drugs, with one targeting agent or multiple targeting agents along with imaging agent etc. Therefore, this type of drug delivery system will have multifunctional activities that will be more effective for the treatment of cancer. Multifunctional nanoparticles (targeting, imaging, sensing, therapy) will be more appropriate for clinical translational.

We believe that in next few years we will see numerous applications of nanotechnology-based therapeutics and diagnostics in clinics. In addition, individualized medicine is another important area where nanotechnology can play a pivotal role. Due to cancer heterogeneity and development of drug resistance any particular targeted therapy may not be effective for every population of patients. Therefore, identification of new molecular markers/targets that will only be present on cancer cells would ideal for nanotechnology based targeted therapy. Similarly, smarter packaging technology is also essential to overcome the challenges posed by the physiologic barrier and by the cancer cells. Thus, basic research both in the field of cancer biology and nanotechnology are essential to meet the challenges that the deadly disease cancer poses to human beings.

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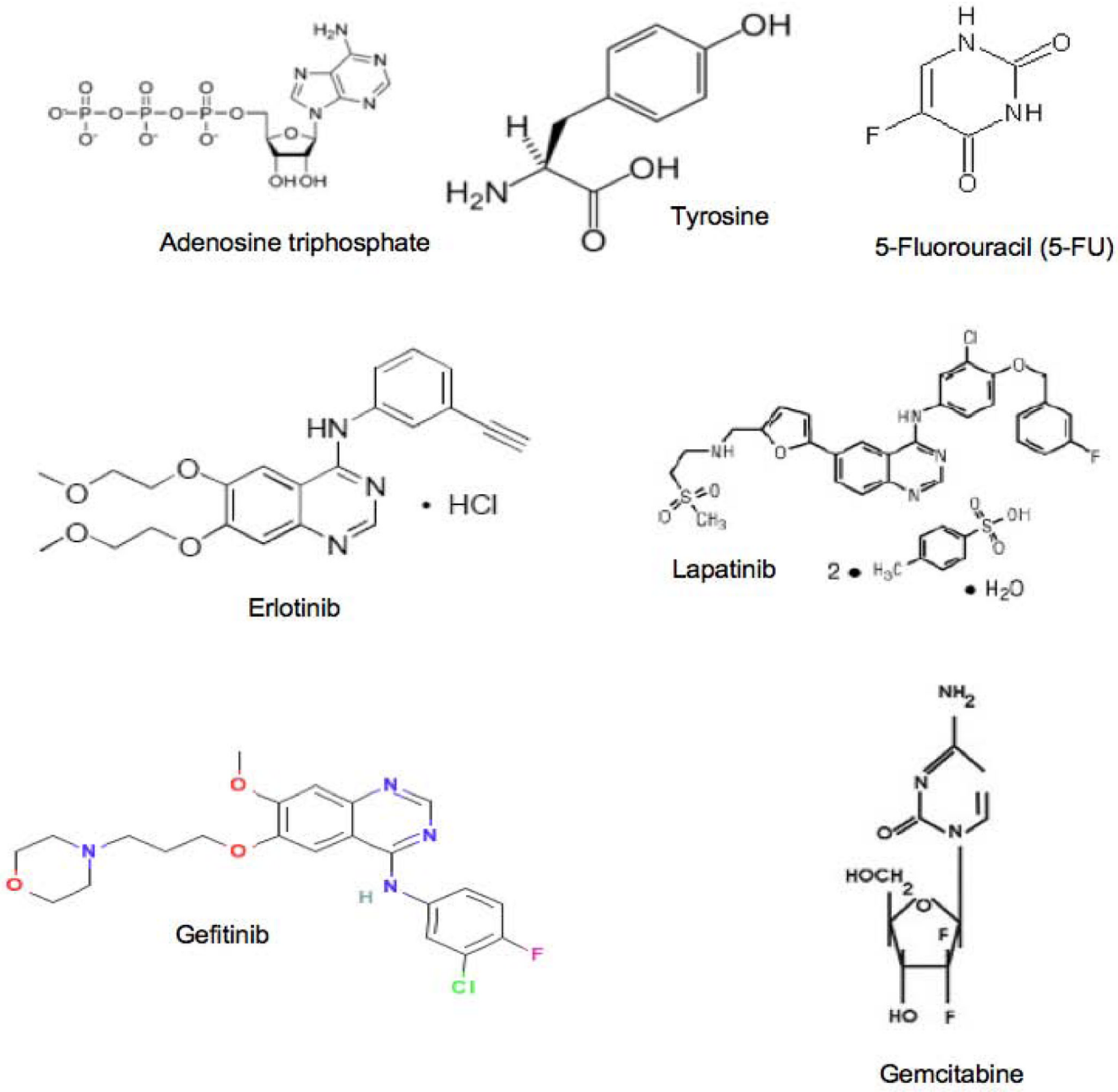

Figure 1.
Chemical formula of some anti-cancer drugs and EMD72000), Trastuzumab (Herceptin)] that inhibit ligand binding to EGFRs and small-molecule tyrosine kinase inhibitors (TKIs).

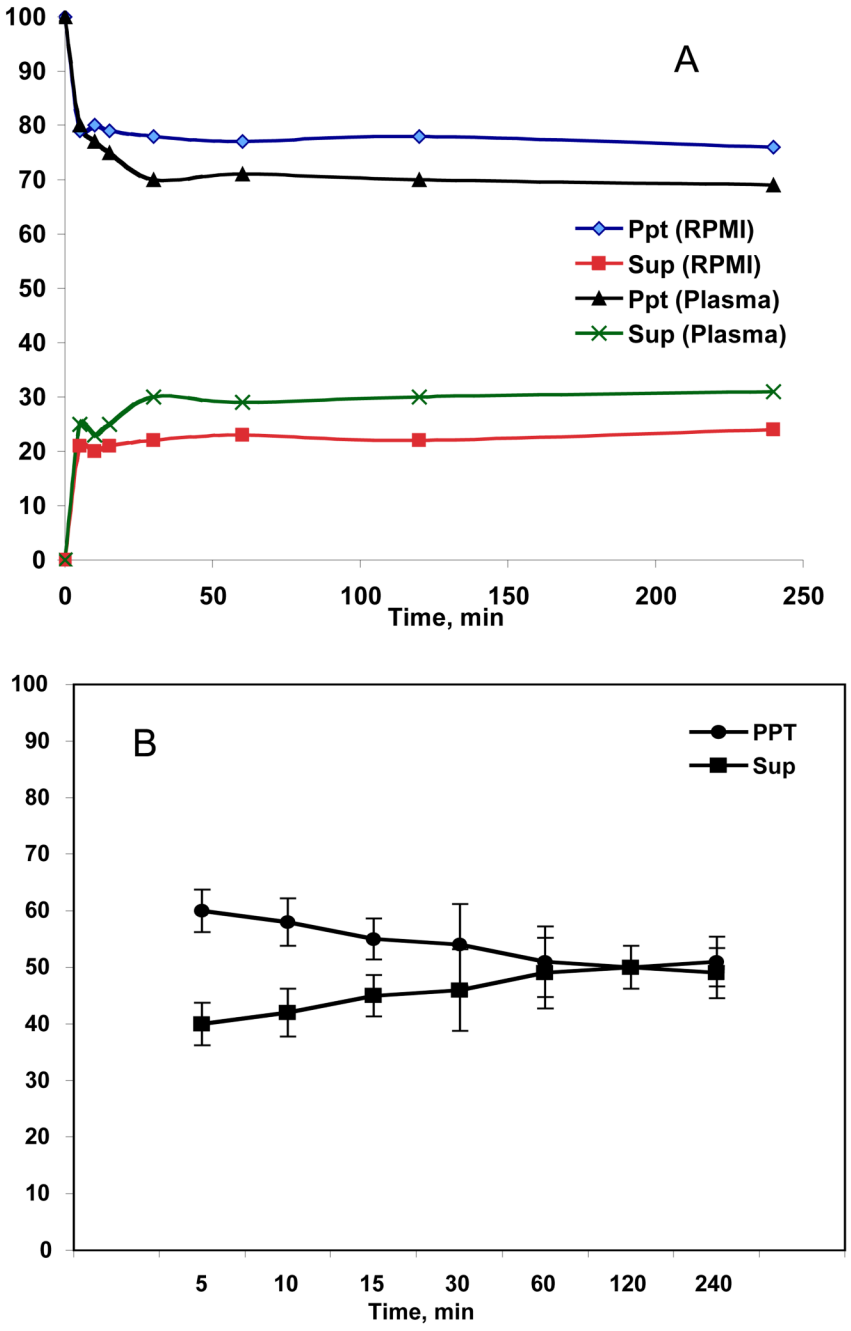

Figure 2.

Release of ^{125}I-C225 and [^{3}H-Gem] from the nanoconjugate in cell growth media mouse plasma. Figure 2A demonstrating the release of C225 from Au- [^{125}I-C225]-Gem in RPMI and in mouse plasma over time. Distribution of ^{125}I-C225 in supernatant and pellet was quantified by radioactivity measurement in a gamma counter. Figure 2B demonstrating the release of 3H-Gem from Au-C225-[^{3}H-Gem] in RPMI and in mouse plasma over time. Distribution of ^{3}H-Gem in the supernatant and in the pellet was quantified by radioactivity measurement in a scintillation counter. Reprinted with permission from Ref. [173], Chitta Ranjan Patra, Sheng Cao, Stephanie Safgren, Resham Bhattacharya, Matthew M. Ames, Vijay Shah, Joel M. Reid, and Priyabrata Mukherjee, Intracellular fate of a targeted delivery system. J. Biomed.

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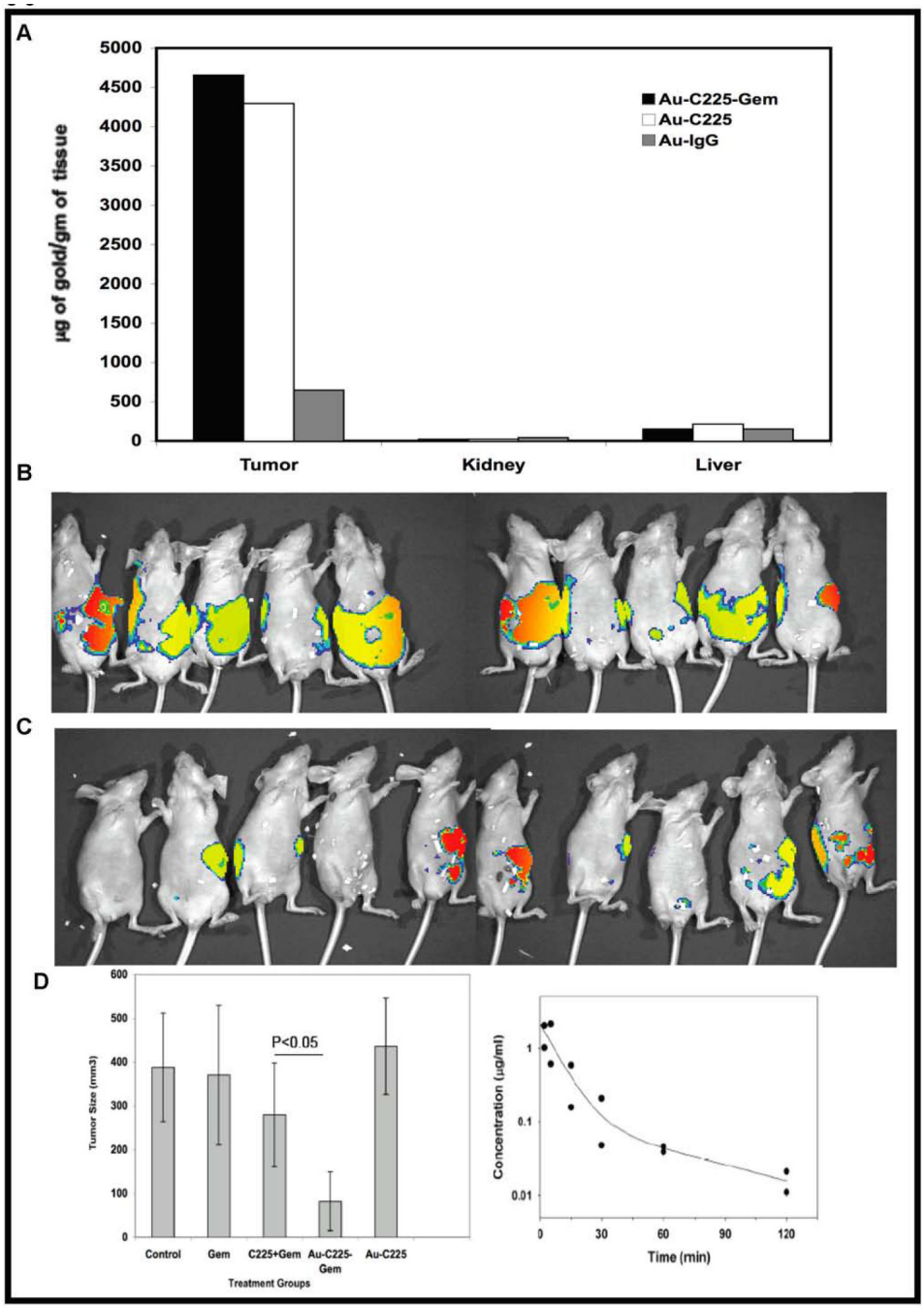

Figure 3.

In vivo targeting of the nanoconjugate and its therapeutic efficacy. A, the quantification of the amount of gold taken up by the tumor, kidney, and liver under different treatment groups (n = 3). A comparative bioluminescence image from the mice treated with a mixture of C225 and gemcitabine (C225 + Gem; B) or Au-C225-Gem (C) i.p. (n = 10). D, effect of different treatment groups on tumor growth inhibition in vivo (left). Tumor volume was measured after sacrificing the mice at the end of the experiment. Right, plasma concentration of gold over time determined by ICP analysis. Blood samples were collected from the mice under isoflurane anesthesia at different time points in heparinized tubes containing tetrahydrouridine to prevent gemcitabine degradation by cytidine deaminase after i.v. drug administration. Reprinted with

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