List of RGJ advisors 2023/2024

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Keywords: Diabetes mellitus, Cholangiocarcinoma, Hyperglycemia, Glycation, Glucose Metabolism

Summary of research:

Epidemiological studies suggest the associations between diabetes mellitus (DM) and cholangiocarcinoma (CCA) in the Northeast of Thailand, where the nationwide highest mortality by both diseased are observed. Our previous reports showed that high glucose conditions in DM promote the progressive phenotypes of CCA, namely, proliferation, migration, and invasion, both in vitro and in vivo. High glucose enhances the aggressiveness of CCA cells via stimulating intracellular signaling pathways and increases abnormal protein glycosylation in the cells, resulting in activated oncogenic pathways. However, the promoting roles of high glucose in CCA progression are not fully understood. Glycation, a non-enzymatic reaction to add sugar groups into other biomolecules, is one of the underlying mechanisms of diabetic complications, while little is known about the roles of glycation in cancer progression. The roles of dietary advanced glycation end products (AGEs) in cancer risk are controversial; endogenous AGEs have been demonstrated for their oncogenic roles in breast cancer, lung cancer, and melanoma, with unclear mechanisms. Our preliminary study also suggests increased AGEs in the extracellular matrix of tumor areas in CCA tissues from patients with DM. The current study is, therefore, proposed to study the biological roles of AGEs in CCA progression. The expression of AGEs and receptors for AGEs (RAGE) will be explored in the sera and tumor tissues of CCA patients with and without DM. The levels of AGEs and RAGEs and the clinicopathological relevance in CCA patients will be examined using an appropriate statistical model. CCA cells cultured in normal and high glucose medium will be used as a model of hyperglycemia-induced AGEs in cancer cells, and cell line-derived xenografts implanted in chemical-induced diabetic immunodeficient mice will be used for the in vivo experiments. Hyperglycemia-induced AGEs in CCA cells will be immunoprecipitated using specific antibodies, and the glycated proteins will be analyzed and identified by proteomic analysis using mass spectrometry. The candidate AGEs and glycated proteins will be validated for their functions in CCA carcinogenesis and progression. The identification of targets and their roles will provide more understanding of the mechanism underlying the associations between DM and CCA, which may help the development of prevention and the improvement of CCA treatment in patients with DM.

แบบเสนอโครงการวิจัย (Research Project)
ประกอบการเสนอขอทุนอุดหนุนการวิจัยของสำนักงานการวิจัยแห่งชาติ (วช.)
โครงการปริญญาเอกกาญจนาภิเษก (คปก.) ภายใต้ความร่วมมือไตรภาคีไทย-สวีเดน
ประจำปีงบประมาณ ๒๕๖๗

๑. ชื่อโครงการวิจัย Roles of advance glycated end products in the progression of cholangiocarcinoma in diabetic conditions

๒. ชื่อ-สกุล อาจารย์ที่ปรึกษา

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๓. กลุ่มสาขาวิทยาศาสตร์พื้นฐานที่สมัคร (เลือกเพียง ๑ กลุ่ม)
☑ ชีววิทยา (Biology) ☐ เคมี (Chemistry)
☐ ฟิสิกส์ (Physics) ☐ คณิตศาสตร์ (Mathematics)

๔. ผู้ใช้ประโยชน์ (Research stakeholders)
☐ มี.....*(โปรดระบุชื่อความร่วมมือ และหน่วยงาน)*.....
☑ ไม่มี

๕. คำสำคัญ (Keyword) ของโครงการ
Diabetes mellitus, Cholangiocarcinoma, Hyperglycemia, Glycation, Glucose Metabolism

๖. ความสำคัญและที่มาของปัญหาที่ทำการวิจัย (Problem statement and significance of research)

Cholangiocarcinoma (CCA), a bile duct cancer, has the globally highest incidence in the Northeast of Thailand. While liver fluke infection is a strong risk factor for people in this area, other factors are proven for their significance in CCA carcinogenesis. Diabetes mellitus (DM), a disease characterized by high blood glucose levels, has a relatively high prevalence and high mortality rate in the same area as CCA. DM and hyperglycemia are the factors that additionally increased the risk of CCA in observational studies and in animal models. People with CCA and DM have a poorer prognosis and shorter survival than those without DM. Our previous studies show that high glucose activates intracellular signaling pathways leading to the higher aggressive phenotypes of CCA cells both in vitro and in vivo. High glucose levels also promote abnormal glycosylation in CCA cells resulting in the increased stability of oncoproteins and transcription factors that regulate the malignant phenotypes. However, physiological dysregulations caused by hyperglycemia in DM are majorly complicated by advanced glycation

end products (AGEs), a non-enzymatic reaction of adding sugar derivative groups to the other biomolecules. There are some reports demonstrating that AGEs and the receptors for AGEs (RAGEs) might play several important roles in cancer progression, but the results are controversial. The discrepancy between the effects of dietary and endogenous AGEs on cancer risk and prognosis is also reported. At present, the understanding of the roles of AGEs in cancer is limited and has never been studied in CCA. The current project is thus proposed to investigate the roles of AGEs in CCA aggression. Understanding hyperglycemia-induced AGEs in CCA will be helpful for the delay of CCA progression and may lead to developing the intervention to improve the prognosis of patients with CCA who have DM.

๗. ทฤษฎี/สมมุติฐานของโครงการ (Hypothesis)

AGEs and RAGEs are increased in CCA under diabetic conditions, which results in higher aggressive phenotypes of CCA cells compared with those in non-diabetic conditions. The intervention to decrease AGEs and RAGEs will help improve CCA treatment in patients with CCA and DM.

๘. วัตถุประสงค์ของโครงการ (Objectives)

1. To investigate the roles of AGEs in CCA progression
2. To identify the potential glycated molecules for CCA-targeted therapy under diabetic conditions

๙. การทบทวนวรรณกรรม/ผลงานวิจัยที่เกี่ยวข้อง (Literature Review)

1. Association between DM and CCA

The association between DM and the increased risk of CCA has been observed by epidemiological studies with unclear mechanisms. Like other cancers, the underlying linkage between DM and CCA is primarily suspected as a result of increased insulin. However, the observational study in insulin-treated individuals found heterogeneity of the effects as exogenous insulin treatment is associated with the increased risk of a particular subtype of CCA. Nowadays, it is widely accepted that the association between DM and cancers, including CCA are multifactorial consequences of DM, in which high glucose is nonnegligible. Our previous studies found that high glucose promotes the aggressive phenotypes of CCA cells, e.g., proliferation, migration, and invasion in vitro and in vivo. High glucose activates JAK2/STAT3 and NF-kB pathways resulting in the transcription of aggressiveness-related genes. High glucose levels also increase abnormal glycosylation, e.g., *O*-GlcNACylation, leading to the stabilization of oncoproteins in CCA cells, resulting in the aggressive progression of the disease.

2. Roles of AGEs in cancer risk

Roles of AGEs and cancer risk may be considered into two categories; dietary and endogenous AGEs. As AGEs can stimulate the inflammation process, it is suspected that dietary AGEs are a

possible risk factor for cancer development. However, a large cohort study reported a null effect on most cancer types and slightly increased the relative risk of prostate cancer. Some specific subgroups of AGEs are associated with an increased risk of specific breast cancer subtypes. Dietary AGEs are associated with an increased risk of gall bladder cancer in one study but is, conversely, associated with a decreased risk of hepatocellular carcinoma. At present, the carcinogenic roles of dietary AGEs and the risk of cancer remain inconclusive.

3. Roles of AGEs in cancer progression

The roles of AGEs in cancer progression is limited and mostly reported in some specific cancer. Studies in breast cancers demonstrated that AGEs activate NF-kB and increased cell proliferation and clonogenicity. AGEs also promote the metastatic potential of breast cancer cells by stimulating the epithelial-mesenchymal transition and increasing the expression of matrix metalloproteinase. The effects of AGEs on breast cancer progression might be a result of RAGEs or Toll-like receptor activation. Moreover, the extracellular matrix of the cancer microenvironment can be abnormally glycated by hyperglycemic conditions. The glycated extracellular matrix can alter the signaling to cancer cells and activate the metastasis of cancer cells. Consistent results are also reported in lung cancers and malignant melanoma. Nevertheless, the study of the roles of AGEs in other cancer is still lacking and need more investigation.

๑๐. ระเบียบวิธีวิจัย (Methodology)

1. CCA cells and tissues

CCA cells used in the project are established from Thai patients. Cells will be cultured in normal and high glucose media to resemble euglycemia and hyperglycemia, respectively. Tumor tissues will be obtained from patients with CCA who underwent surgical resection at Srinagarind Hospital, Khon Kaen University. Written informed consent will be obtained from all patients.

2. AGEs and RAGEs profiling

The effects of hyperglycemia-induced AGEs in CCA will be investigated both in CCA cells and CCA tissues from patients using specific antibodies by Western blots, immunocytofluorescence, and immunohistochemistry. The comparison between the diabetic and non-diabetic groups will be analyzed using an appropriate statistical model.

3. Roles of AGEs and RAGEs in CCA progression

Roles of AGEs and RAGEs in CCA progression will be studied using in vitro model. Hyperglycemia-induced AGEs will be generated using high glucose and chemical reactions following available methods in the literature. Purified AGEs will be treated to CCA cells, and aggressive phenotypes, e.g., proliferation, clonogenicity, migration, and invasion, will be

determined and compared between the AGEs-treated group and control. Roles of RAGEs in CCA progression will also be investigated using siRNA or shRNA to knock down its expression.

4. In vivo model for DM-induced AGEs in xenografted CCA and roles of RAGEs in CCA progression

The xenografted CCA model will be conducted by; firstly, inducing immunodeficient mice to have DM using streptozotocin, secondly implanting human CCA cells into the mice and allowing xenografted CCA to grow in an appropriate timeframe. The control mice without DM will be implanted with the same cell line but without DM induction. CCA tumors from DM and non-DM mice will be profiled for the AGEs expression and analyzed for the correlation with blood glucose level and the tumor burden. Roles of RAGEs in CCA progression will be investigated by stable knockdown using shRNA of RAGEs in CCA cells and then be implanted into DM and non-DM immunodeficient mice. Parental cells with scramble shRNA will also be as the control group. All the in vivo experiments will be followed the regulations of the Institute Animal Care and Use Committee.

5. Identification of the glycated proteins of AGEs and their roles in CCA

The target proteins of glycation in CCA will be identified using proteomic analysis. All glycated proteins will be immunoprecipitated by specific antibodies to AGEs from the xenografted CCA tumors from DM and non-DM mice. Proteomics will be done using mass spectrometry. A list of significant glycated proteins will be selected for a further study of protein post-translational modification by glycation.

๑๑. ขอบเขตของการวิจัย (Scope of the study)

The study of the roles of AGEs and RAGEs in the present project will be done in vitro and in vivo using liver fluke-associated CCA cells and tissues, which may have different genetic backgrounds from non-liver fluke-associated CCAs. Diabetic conditions in the study will be mainly focused on the impact of high glucose levels rather than high blood insulin.

๑๒. ผลผลิต (Output) ผลลัพธ์ (Outcome) และ ผลกระทบ (Impact) ที่คาดว่าจะได้จากการวิจัย

Output: At least one publication in a Scopus-indexed Q1 journal and one PhD graduate

Outcome: A new knowledge of the associations of DM and CCA

Impact: Increase awareness of DM complications and the effects of high blood glucose on CCA progression, and suggest guidance to develop the treatment and care of patients with CCA and DM

กรุณาจัดทำเป็นภาษาอังกฤษ เนื่องจากฝ่ายสวีเดนจะเป็นผู้พิจารณาข้อเสนอ
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