The Apoptosis Induction Effect of Metformin in Human Pancreatic Cancer

**Introduction:**

Pancreatic cancer is one of the deadliest cancers all over the world. Current treatment methods for pancreatic cancer are limited and often ineffective. This indicates the necessary of developing new and effective therapies. Metformin, a widely used medication for type 2 diabetes treatment, has recently gained attention for its potential anti-cancer properties. This research aims to study the apoptosis induction effect of metformin in human pancreatic cancer.

**Hypothesis:**

Metformin has an apoptosis induction effect on human pancreatic cancer cells.

**Material list:**

**Drugs for analysis:**

1. Synthesized drug: Metformin (for treatment group)

(In the market, it is usually 500 mg/tab. Dilutions: from 0.25 to 2 g/L)

1. Positive control drug: 5-fluorouracil (for positive control)
2. Negative control drug: DMSO (for negative control)

Notes: Metformin is a very common diabetic drug. For 5-fluorouracil, other tumor suppressor drugs are also ok. If there are too many sets of experiment, we can cancel the positive control.

**Cell Culture:**

1. Pancreatic cancer cell line: AsPC-1 <https://www.atcc.org/products/crl-1682>

(Sufficient number of cells e.g., 1-2 million cells per sample for protein extraction)

1. Non-cancerous cell line: hTERT-HPNE <https://www.atcc.org/products/crl-4023>

(Sufficient number of cells e.g., 1-2 million cells per sample for protein extraction)

1. Culture medium: RPMI-1640 (for cancer cell line); DMEM/F12 (for non-cancerous cell line)

(Sufficient volume for cell culture and maintenance throughout the experiment)

1. Fetal bovine serum (FBS)

(As manufacturer's recommendation, typically 10-20% (v/v) in culture media)

1. Trypsin-EDTA

(Sufficient volume for cell detachment)

1. Penicillin-streptomycin solution

(As manufacturer's recommendation, typically 1-2% (v/v) in culture media)

1. Culture plates
2. Washing solution: PBS buffer

(Sufficient volume for cell washing and buffer preparation)

Notes: Pancreatic cancer cell line is less common. It is ok if other types of cancer cell line are provided. But please provide me a corresponding normal cell line:-)

**Cell Viability Assay: MTT assay**

1. 96-well plates
2. Cell viability assay kit: MTT assay kit, including MTT reagent, Solubilization Solution, and so on.

(As manufacturer's recommendation, typically 5 mg/mL stock solution)

**Annexin V/PI Apoptosis Assay: Flow Cytometry**

1. Flow cytometry reagents: Annexin V apoptosis detection kit, including Annexin V-FITC conjugate, PI solution, binding buffer (HBSS)

(More than 6 ml staining solution should be used)

1. Flow cytometer with appropriate lasers and detectors
2. Statistical software

**Caspase Apoptosis Assay: Western Blot**

1. Cell lysis buffer: RIPA buffer

(Sufficient volume, typically 1-2 ml per sample)

1. Protease inhibitor cocktail

(As manufacturer's recommendation, typically 1x concentration in protein extraction buffer)

1. Phosphatase inhibitor cocktail

(As manufacturer's recommendation, typically 1x concentration in protein extraction buffer)

1. Protein quantification kit: BCA protein assay kit

(Sufficient for protein quantification)

1. SDS-PAGE gel

(For 10% Separating gel: 4 ml Stacker A (30% Acr-Bis) + 4 ml Stacker B (Tris-HCl mixed with 10% SDS) + 40 ul 10% APS + 4 ul TEMED)

1. Electrophoresis machine

(Can work normally)

1. Tris-glycine running buffer

(Sufficient volume for gel electrophoresis and buffer preparation)

1. PVDF membrane

(Sufficient size)

1. Transfer buffer

(28.8 g of glycine + 6.04 g of tris-base + 200 ml ethanol and complete the volume to 1L with dH2O)

1. Blocking buffer

(0.1%v/v Tween 20 in tris-buffer saline (TBS))

1. Primary antibody against target protein (to target caspase 3 or 8)

(As manufacturer's recommendation, typically 1:1000 to 1:2000 dilution)

1. Secondary antibody conjugated to HRP

(As manufacturer's recommendation, typically 1:100,000 to 1:250,000 dilution)

1. Chemiluminescent substrate for HRP

(As manufacturer's recommendation, typically 50 ml for 1000 cm2)

1. Washing buffer

(Sufficient volume of TBS: 100 mM Tric-HCl, pH 7.8 + 0.9 w/v NaCl)

Notes: I am not sure whether the material that I listed for Western Blot is sufficient. But I learned that our lab has already had Western Blot set to detect caspase:-)

**Notes:** Each set of the experiment is duplicate. Also, the amount of material listed is just a reference:-)

Hope that sufficient number of materials are prepared. Thank you so much ~