Chapter 28
Heterogeneity of Mitochondrial Redox State in Premalignant Pancreas in a PTEN Null Transgenic Mouse Model

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Abstract Pancreas-specific deletion of PTEN in mice revealed progressive premalignant lesions such as ductal metaplasia with infrequent malignant transformation. In this study, we aimed at evaluating the mitochondrial redox state of the metaplastic pancreas in a pancreas-specific PTEN null transgenic mouse model. The two intrinsic fluorophores, reduced nicotinamide adenine dinucleotide (NADH) and oxidized flavoproteins (Fp) such as flavin adenine dinucleotide (FAD), in the respiratory chain in mitochondria are sensitive indicators of mitochondrial redox states and have been applied to the studies of mitochondrial function with energy-linked processes. The redox ratio, Fp/(Fp+NADH) provides a sensitive index of mitochondrial redox state. We have obtained optical images of the in vivo mitochondrial redox states of the snap-frozen pancreases from pancreas-specific PTEN null mice (Pdx1-Cre;PTEN\textsuperscript{lox/lox}, N=3) and the controls (PTEN\textsuperscript{lox/lox}, N=3) using the redox scanner at low temperature. The results showed high spatial heterogeneity of mitochondrial redox state in the mutated pancreases with hot spots of much higher Fp redox ratios whereas the normal ones, were relatively homogenous. The cystic dilation regions in the metaplastic pancreases showed little to no NADH or Fp signal. Histological analysis confirmed no cells existed in these regions. It is the first time that the in vivo mitochondrial redox states of the metaplastic mouse pancreas were optically imaged. Our previous results on human melanoma and breast cancer mouse xenografts have shown that mitochondrial redox state quantitatively correlates with cancer metastatic potential. The more oxidative mitochondrial redox state (higher Fp redox ratio) corresponded to the higher metastatic potential of the tumors. As mitochondrial redox state imbalance is associated with abnormal mitochondrial function, and redox state mediates the generation of reactive oxygen species and many signal transduction pathways,

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this research may provide insights for studying basic biology and developing early
diagnostic imaging biomarkers for pancreatic cancer.

28.1 Introduction

Early detection of pancreatic cancer demands reproducible imaging biomarkers. Pancreas-specific deletion of PTEN in mouse revealed progressive premalignant lesions such as ductal metaplasia with infrequent malignant transformation [1]. PTEN is a tumor suppressor gene inhibiting the activity of PI3K/Akt signaling pathway, which plays a key role in cancer progression. This premalignant pancreatic cancer model provides an excellent tool for searching for biomarkers in early pancreatic cancer detection. In mitochondria, the two intrinsic fluorophores, reduced nicotinamide adenine dinucleotide (NADH) and oxidized flavoproteins (Fp) such as flavin adenine dinucleotide (FAD), in the respiratory chain are sensitive indicators of mitochondrial redox states and have been applied to the studies of mitochondrial function with energy-linked processes. The redox ratio, Fp/(Fp+NADH) provides a sensitive index of mitochondrial redox [2-6].

Previously, by using the low-temperature redox scanning technique [6-9] we discovered that in vivo mitochondrial redox state is a sensitive marker distinguishing between normal tissue and human melanoma xenografted in mice [10] and differentiating tumor aggressiveness among five human melanoma tumor lines spanning a wide range of metastatic potential in mouse xenografts [11]. In the present investigation, we report the preliminary results of quantitative mitochondrial redox imaging of mouse pancreases using the pancreas-specific PTEN knockout mice as the model system. The possible link between the premalignant lesions in the pancreas and the in vivo mitochondrial redox state, provides insights for basic biology studies and may aid the development of early diagnostic imaging biomarkers for pancreatic cancer.

28.2 Methods

Three PTEN null mice (Pdx-1-Cre;PTEN$^{lox/lox}$) and three control mice (PTEN$^{lox/lox}$) were prepared in the Stanger laboratory [1]. All mice were about 8 months old. The pancreases of the anesthetized mice were quickly resected and dipped into liquid nitrogen within 2 seconds after removal. The samples for redox scan were prepared as previously described [12, 13]. Briefly, a snap-frozen pancreas was carefully placed into the chilled mounting medium composed of ethanol-glycerol-water (10:30:60) contained in a plastic screw closure of 24 mm diameter. Frozen reference standards (one NADH and one Fp with known concentrations) were quickly mounted adjacent to the tissue. The screw closure containing the tissue and reference standards was then maintained in liquid nitrogen awaiting redox scanning.