MicroRNA Regulation of Cholesteatoma Growth
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ABSTRACT

Objective: Identify novel regulatory mechanisms controlling growth and proliferation of cholesteatoma. Specifically, the potential role of microRNAs and downstream proteins has been studied in cholesteatoma.

Methods: Cholesteatoma and normal skin were taken from patients at the time of surgery. Tissue was processed for RNA and protein extraction. Real-time RT-PCR was used to assess levels of human microRNAs. Western blot analysis was used to assess levels of upstream and downstream regulatory proteins.

Results: Several microRNAs were found to be up-regulated in cholesteatoma as compared to normal skin, especially hsa-mir-21 (hsa-mir-21), which has been associated with numerous other human neoplasms. Further characterization of hsa-mir-21 showed a greater than 4-fold higher expression in cholesteatoma. The downstream targets of hsa-mir-21, PTEN and PDCD4, were found to be reduced in cholesteatoma compared to normal skin.

Conclusions: Hsa-mir-21 causes downregulation of the tumor suppressor gene PTEN which regulates apoptosis, proliferation, invasion and angiogenesis. The results of this study are consistent with this model of regulatory interaction between hsa-mir-21 and proteins PTEN and PDCD4.

INTRODUCTION

Cholesteatomas are benign, but aggressive, lesions typically found in the outer and middle ears. Epidemiologically derived, they are more accurately termed keratinomas and represent dysregulated keratinocyte growth. Cholesteatomas may be congenital or secondarily acquired and have an incidence of approximately 6–10 per 100,000 population (Kompf et al., 1996; Homoe, 2002). Despite advances in medical therapy, such as aminoglycoside antibiotics and nasal steroid sprays (Rosberg, 2007), cholesteatoma is a chronic relapsing disease with the risk of regrowth.

Methods and Results

ABSTRACT

METHODS AND RESULTS

OVERALL MODEL

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DISCUSSION

MicroRNAs represent powerful regulators of protein translation, and their dysregulation has been implicated in many neoplastic diseases. This study specifically identified up-regulation of hsa-mir-21 and down-regulation of the target tumor suppressor proteins PTEN and PDCD4 in cholesteatoma. These proteins control aspects of apoptosis, proliferation, invasion, and migration. The results therefore define a model potentially explaining cholesteatoma growth through microRNA dysregulation.

These data help to formulate our model of hsa-mir-21 up-regulation in epithelia, which leads to PTEN and PDCD4 expression and subsequent cholesteatoma formation. We posit that hsa-mir-21 up-regulation is secondary to infection and tissue cytokine production. It has been shown that lipopolysaccharides from bacterial infection can stimulate cytokine production (Park et al., 2003). Specifically, lipopolysaccharide activates IL-6, IL-1 and TNF-alpha production (Park et al., 2003). These factors have been shown to be up-regulated in cholesteatoma (Kane et al., 1996; Wilmer et al., 2003; Meltz et al., 2007; Schmidt et al., 2001, Alkino et al., 2009, Buij et al., 1996). Most important, these activation events in IL-6, which increase microRNA expression, target mimicry of hsa-mir-21-mediated suppression of PTEN, PDCD4 and TPM1, leading to keratinocyte proliferation, migration, growth, and invasion.