Blastocystis sp. subtype 3 triggers higher proliferation of human colorectal cancer cells, HCT116

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Abstract Blastocystis sp is a commonly found intestinal microorganism and was reported to cause many nonspecific gastrointestinal symptoms. Various subtypes have been previously reported, and the pathogenicity of different subtypes of Blastocystis is unclear and remains as a controversial issue. A recent study has shown that the Blastocystis antigen isolated from an unknown subtype could facilitate the proliferation of colon cancer cells. Current study was conducted to compare the effect of solubilized antigen isolated from five different subtypes of Blastocystis on colon cancer cells, HCT116. A statistically significant proliferation of these cells was observed when exposed to 1.0 μg/ml solubilized antigen isolated from subtype 3 Blastocystis (37.22%, p<0.05). Real-time polymerase chain reaction demonstrated the upregulation of Th2 cytokines especially transforming growth factor beta in subtype 3-treated cancer cells (p<0.001; 3.71-fold difference). Of interest, subtype 3 Blastocystis antigen also caused a significantly higher upregulation of cathespin B (subtypes 1 and 2, p<0.01; subtypes 4 and 5, p<0.001; 6.71-fold difference) which lead to the postulation that it may enhance the exacerbation of existing colon cancer cells by weakening the cellular immune response. The dysregulation of IFN-γ and p35 expression also suggest Blastocystis as a proponent of carcinogenesis. Therefore, it is very likely for subtype 3 Blastocystis to have higher pathogenic potential as it caused an increased propagation of cancer cells and substantial amount of inflammatory reaction compared to other subtypes.

Introduction

Blastocystis is an anaerobic microorganism which is commonly found in the human stool sample (Winder et al. 2002). It is known to cause many nonspecific symptoms such as diarrhea, abdominal pain, and flatulence (Suresh et al. 2009) which may be observed in immunocompromised and immunocompetent hosts (Doyle et al. 1990; Giromini et al. 1999). High rates of Blastocystis infection are found in developed countries (Wong et al. 2008). To date, nine different subtypes of Blastocystis have been reported in human based on genomic studies (Yoshikawa et al. 1998, 2000, 2004). The pathogenesis of Blastocystis remains as a controversial issue, as it cannot be clearly assigned to certain genotype. Recently, the solubilized antigen of Blastocystis was shown to facilitate the in vitro proliferation of human colorectal carcinoma cells (HCT116) (Chandranath et al. 2010). It was also observed to cause oxidative damage in rats inoculated with human-derived Blastocystis isolate (Chandranath et al. 2009). However, these studies were limited to only one single isolate. Therefore, the present study attempts to evaluate the effect of solubilized antigen isolated from five different subtypes of Blastocystis on colon cancer cells, HCT116 proliferation. In addition to that, we also compared the gene expression of cytokines, nuclear transcription factors, and apoptotic genes (Table 1) in colon cancer cell line in the presence of Blastocystis (Figs. 1, 2, 3, and 4).

Methods

Preparation of solubilized antigen from Blastocystis

The axenic Blastocystis was collected using the Ficoll-Paque density gradient centrifugation method as described