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Activation of anti-apoptotic machinery downstream to Fas/FasL pathway in primary mixed and pure mucin producing cholangiocarcinoma cells: key role of c-FLIP

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Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignancies lacking of effective strategies for prevention and cure. Recently, we have established a protocol for the isolation of primary cells from mixed and mucin specimens of human CCA. To this regard, the aim of this study was to analyze the influence on proliferation and apoptosis as well as the related modifications of apoptotic machinery downstream to Fas/FasL pathway in primary cultures of human mixed and mucin-producing CCA after direct co-culture with peripheral blood mononuclear cells (PBMCs). Our findings show that both IH-CCA subtypes constitutively express high levels of Fas and FasL. Following direct co-culture with PBMCs, the expression of Fas and FasL significantly increased after 24, 48 and 72 hours of exposure ($p< 0.05$). At the same time, a significant increase of percentage of apoptotic CD4+ and CD8+ T-cells or Natural Killer CD56+ cells was observed along the co-cultures compared to PBMCs cultured alone ($p<0.05$). Conversely, both IH-CCA subtypes showed an augmentation of the proliferation rate after co-culture with PBMCs ($p<0.05$). WB analysis revealed a stable expression of FADD in IH-CCA primary cells either cultured alone or co-cultured with PBMCs. Interestingly, both IH-CCA subtypes showed an increased expression of c-FLIPS/L, namely, a 47±3% increase in mucin CCA co-cultured for 24 hours with PBMCs vs cells cultured alone ($p< 0.05$), and a 35±3% increase in mixed CCA co-cultured for 24 hours with PBMCs vs cells cultured alone ($p< 0.05$). IF analysis showed a strictly nuclear staining for c-FLIPS/L in both IH-CCA subtypes cultured alone, whereas, after co-culture with PBMCs, either a nuclear and a cytoplasmic staining for c-FLIPS/L were observed. Interestingly, a significant increase of the expression of pro-caspase 8 and Bcl-2 was detected. In conclusion, these data demonstrate that a direct co-culture with PBMCs induces an increased expression of Fas and FasL, followed by an increase of c-FLIPS/L in primary cultures of mixed and mucin IH-CCA, culminating in anti-apoptotic and proliferative effects in cancer cells. Moreover, as shown for other cancer cells, c-FLIPS/L proved to be the key molecule of cell proliferation and survival in the immune-escape in subtypes of IH-CCA and might represent a potential therapeutic target in deadly and drug refractory cancers.

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