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Conditioned medium from stimulated macrophages inhibits growth but induces an inflammatory phenotype in breast cancer cells

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ABSTRACT

Disparate roles exist for tumor-associated macrophages in breast cancer growth and progression. The aim of this study was to explore the influence of induced macrophages on the growth of breast cancer cells. THP-1 monocytes were differentiated to macrophages using phorbol 12-myristate 13-acetate. The effect of the medium from THP-1 monocytes or macrophage-conditioned medium (MφCM) on MCF-7 (estrogen receptor and progesterone-positive positive) and MDA-MB-231 (MB; triple-negative) breast cancer cells was determined at 24 h, 48 h and 72 h. Assays were conducted for cell viability, apoptosis, proliferation and cell phenotype, and quantitative real-time polymerase chain reaction (qRT-PCR) for expression of associated genes. MφCM inhibited proliferation of MCF-7 and MB cells in a time-dependent manner and, in particular, decreased viability of MCF-7 cells. MφCM induced a markedly vacuolated phenotype in MCF-7 increased apoptosis in MCF-7 cells, but correlative changes in Bcl-2 or Bax were absent. A multifold and significant reduction in anti-apoptotic Bcl-2 in MB cells was not matched by increased apoptosis. The cell cycle inhibitor CDKN1A was increased in both cell lines, but PCNA decreased only in MB cells. Senescence-associated galactosidase beta-1 (GLB1) mRNA was decreased in MCF-7 cells (48 and 72 h) but increased in MB cells (72 h). Increased expression of interleukin-6 (IL-6) and IL-8 was seen in both cell lines, and increased tumor necrosis factor- α was seen at 24 h for MB and 72 h for MCF-7 indicating increased inflammatory responses of the cancer cells. The two breast cancer celllines had different responses to MφCM, mainly involving inhibition rather than stimulation of growth of the cells, stimulation of senescence (MB cells) and increased inflammatory cytokine expression. The estrogen and progesterone receptor status of the cell lines may determine their response to MφCM. The function of the inflammatory cytokines in breast cancer growth remains to be identified.

1. Introduction

Breast cancer is one of the most common malignancies affecting women. With better treatments and an emphasis on early detection in the past decades, the mortality of breast cancer has decreased steadily since 1990 [1], however it is still the second leading cause of all cancer-related deaths in women worldwide, with recurrence and distant metastasis major reasons for poor clinical outcomes in breast cancer patients [2]. The tumor microenvironment is now recognised as having a major role in cancer progression. Malignant cells evade normal lymphocytic immune surveillance and secrete molecules that establish an environment reminiscent of wound healing, but with a population of macrophages, called tumor-associated macrophages (TAMs), that now have the ability to stimulate growth and invasiveness of the cancer cells [3–5]. Breast cancer has a complex tumor microenvironment that includes perivascular cells, fibroblasts, adipocytes, and highly active TAMs [5]. One of the benefits of defining the presence and role of TAMs in breast cancer pathogenesis is that they may be targeted by novel

Abbreviations: MφCM, conditioned medium of macrophages; PMA, phorbol 12-myristate 13-acetate; MTT, 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl-tetrazolium bromide; qRT-PCR, quantitative real-time polymerase chain reaction; TAM, tumor associated macrophage; DMSO, dimethyl sulfoxide; Bcl-2, B-cell lymphoma-2; PCNA, proliferating cell nuclear antigen; GLB1, galactosidase beta 1; CDKN1A, cyclin-dependent kinase inhibitor 1A; IL-8, interleukin 8; IL-6, interleukin 6; TNF-α, tumor necrosis factor- alpha

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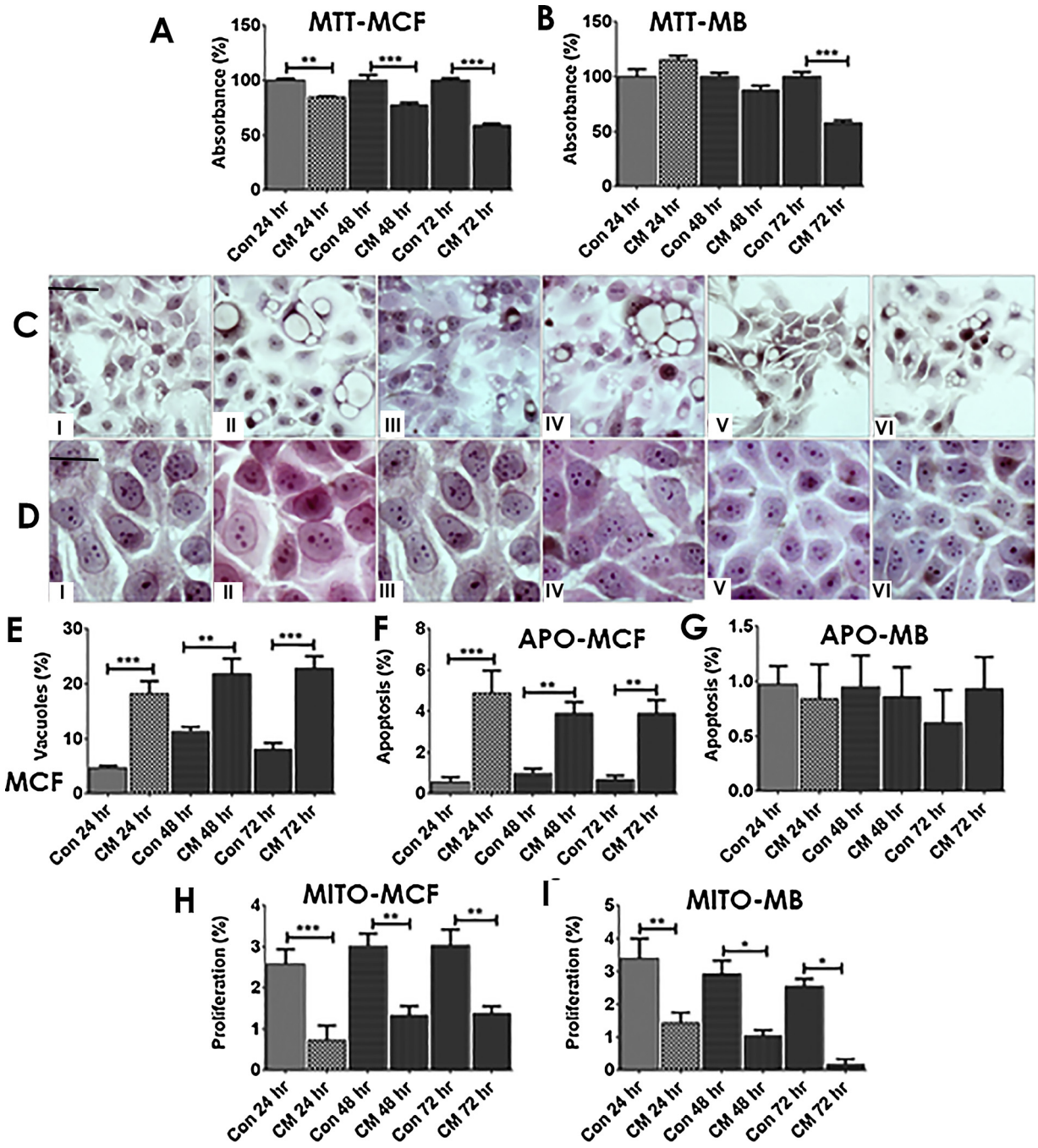

Fig. 1. Growth characteristics of MCF-7 (MCF) and MDA-MB-231 (MB) cells.
MTT assays (Fig. 1A, B) demonstrated decreasing viability of MCF cells in a time-dependent manner after treatment with MφCM, and in contrast, MB cells maintained viability in the first 48.h, then viability decreased at 72 h. The histopathology of MCF is demonstrated in row C, and of MB in row D. For each cell type, the control and MφCM -treated cells are shown in pairs for 24 h (C I, C II; D I, D II), 48 h (C III, C IV; D III, D IV) and 72 h (C V, C VI; D V, D VI). Marked vacuolation was seen in treated MCF-7 cells at all time points (summarized in Fig. 1E). Graphs for % apoptosis are shown as APO-MCF (Fig. 1F) and APO-MB (Fig. 1G). They demonstrate increasing apoptosis in MCF cells but not MB cells. Graphs for % cell proliferation are shown as MITO-MCF (Fig. 1H) and MITO-MB (Fig. 1I). They demonstrate that all MφCM-treated cultures had significantly decreased cell proliferation. Scale bar in row C = 30 μm; scale bar in row D = 10 μm. All photos in row C are the same magnification, and all in row D are the same magnification. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

therapies that, for example, use nanoparticles embedded with anti-macrophage product drugs with high anti-tumor efficiency [6].

Inflammation is a major hallmark of cancer [7,8], with macrophages the most abundant cellular component of the tumor microenvironment. In breast cancers, they comprise up to 50% of the cell mass [9]. The role of macrophages in the progression of breast cancer is still controversial, although most clinical studies have shown that there is correlation between higher density of macrophages in the tumor mass and increased angiogenesis, low estrogen receptor (ER) and progesterone receptor (PR) status, poor prognosis and higher risk of distant metastasis in breast cancer patients [10–12]. In contrast, no significant difference in overall patient survival was found compared with density of macrophage infiltration in T1 stage ER positive (ER+) breast cancer [13]. In addition, Mahmoud et al. demonstrated that CD68 positive macrophages in tumor tissue were not related to prognosis of breast cancer patients [14]. The role of TAMs in different types of anti-cancer