

Production of P-glycoprotein from the *MDR1* upstream promoter is insufficient to affect the response to first-line chemotherapy in advanced breast cancer

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Multidrug resistance, the phenomenon by which cells treated with a drug become resistant to the cytotoxic effect of a variety of other structurally and functionally unrelated drugs, is often associated with the expression of P-glycoprotein, an efflux membrane pump coded by the *MDR1* (*ABCB1*) gene. Transcription from *MDR1* can start at 2 promoters: a well-characterized downstream promoter and an as yet uncharacterized upstream promoter (USP). We have previously determined that the USP is activated in some drug-resistant cell lines, in primary breast tumors and in metastatic epithelial cells isolated from the lymph nodes of breast cancer patients. In this study, we report the cloning and characterization of the *MDR1* USP and studied its association with chemotherapy response in breast cancer patients. Deletion analysis indicated that a nearby endogenous retroviral long terminal repeat is not responsible for promoter activation, and that the region within the first 400 nucleotides upstream from the transcription start point contained all the elements necessary for promoter activity in drug-resistant cells. We identified an element recognized by the transcription factor NF-IL6 (activated upon interleukin-6 exposure) which is necessary for promoter activity in drug-resistant cells and plays a role in the activation of the promoter in response to interleukin-6 in breast cancer MCF-7 cells. Although transcripts from this promoter are associated with translating polyribosomes, their low abundance makes the amount of synthesized P-glycoprotein insufficient to affect the response to first-line chemotherapy in patients with advanced breast cancer.

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The *MDR1* (*ABCB1*) gene codes for P-glycoprotein, an ATP-dependent membrane transporter which pumps many cytotoxic drugs out of the cells and confers resistance to chemotherapy.^{1,2} Efforts to circumvent the occurrence of drug resistance in the clinic have focused mainly on the development of P-glycoprotein modulators³ and *MDR1* transcriptional repressors.^{4,5} Transcription from *MDR1* can start at 2 promoters: a major downstream promoter (DSP), which is used by most cell lines and tissues expressing the *MDR1* gene,⁶ and a minor upstream promoter (USP).^{7–9} Although lacking a TATA-box, the DSP promoter has a CAAT box and an inverted CCAAT element/Y-box upstream from the transcriptional start site (tsp), where an initiator element (Inr) is found. Reporter gene expression studies have identified an array of transcription factor binding sites including, among others, 2 GC boxes, 2 p53 elements, an ETS-binding element, a heat shock element, several T-cell factor elements, a nuclear factor for interleukin-6 (NF-IL6) element and an activator protein-1 site.¹⁰ In addition, a region termed the enhanceosome has been identified in the *MDR1* DSP at which different stress signals converge to upregulate *MDR1* transcription.¹¹ Several stimuli, including histone deacetylase inhibitors, differentiation agents, ultraviolet irradiation and the chemotherapeutic drug doxorubicin, mediate the binding of an enhanceosome complex containing the trans-acting factors, NF-Y, Sp1 and Sp3, the recruitment of the histone acetyltransferase P/CAF to this complex, histone acetylation and chromatin remodeling, thus promoting *MDR1* transcription.¹²

In acute lymphoblastic leukemia patients overexpressing P-glycoprotein, the *MDR1* USP represents the major promoter (and in some patients, the only promoter) used by mononuclear cells.¹³ In breast cancer, the presence of transcripts derived from the *MDR1* USP correlates with metastatic invasion of lymph nodes, and can be detected in isolated carcinoma cells, from both the primary tumor and from invaded lymph nodes.¹⁴ However, the *MDR1* USP has not been characterized in detail. Here we report the detection of tissue-specific *MDR1* USP-derived transcripts in normal human tissues, the characterization of this promoter in drug-resistant cells and its activation by the interleukin-6 (IL6)/NF-IL6 pathway. Because of their low abundance, translation of P-glycoprotein from *MDR1* USP-derived transcripts is insufficient to affect the response to first-line chemotherapy in patients with advanced breast cancer.

Material and methods

Patients and tumors

A total of 60 patients with classical, Philadelphia chromosome positive chronic myelogenous leukaemia (CML) seen at the Hematology Department, Hammersmith Hospital, were used in this study. Peripheral blood samples were taken from the patients, after informed consent, at presentation in chronic phase. Only 1 patient, who showed activation of the *MDR1* USP, had been previously treated and was resistant to busulfan therapy. White blood cells were separated from the whole blood by red cell lysis.

A total of 137 primary breast cancer tissues from patients who developed recurrent disease, and were treated with first-line chemotherapy, were analyzed. After analysis of housekeeping mRNAs for normalization of quantitative PCR (see later), 22 were discarded (20 due to an aberrant *RPL0:GAPDH* ratio and other 2 due to an ambiguous chemotherapy response). The patient and tumor characteristics of the remaining 115 patients in association with

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Abbreviations: CI, confidence interval; CML, chronic myelogenous leukemia; DSP, downstream promoter; IL6, interleukin-6; Inr, initiator element; IVS, intervening sequence; LTR, long terminal repeat; NF-IL6, nuclear factor for interleukin-6; OR, overall response; PBMCs, peripheral blood mononuclear cells; PFS, progression-free survival; tsp, transcriptional start site; uORF, upstream open reading frame; USP, upstream promoter.

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