Advances in Brief

Human Melanoma Cells Transcribe Interleukin 1 Genes Identical to Those of Monocytes

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Abstract

Two molecular species of the pleotropic cytokine interleukin 1 (IL-1) are produced as products of two distinct genes transcribed by cells of the monocyte-macrophage lineage. We have shown previously that a significant proportion of human melanoma cell lines express IL-1 biological activity, but it has not been demonstrated that this activity is the same as authentic monocyte IL-1α and -1β. Here we report the cloning and sequencing of IL-1 complementary DNAs from a metastatic melanoma cell line and demonstrate that they encode bona fide IL-1α and IL-1β. In addition, IL-1 complementary DNAs encoding a different amino acid at position 145 were revealed.

Introduction

Cultured melanoma cells have been reported to produce a variety of growth factors and cytokines (1, 2) which may regulate tumor growth, modify adjacent mesenchyme, or modulate tumor immunity. IL-1 is a potent inflammatory mediator and growth regulator composed of at least two proteins, IL-1α and IL-1β (reviewed in Ref. 3), and is produced by monocytes. In addition, diverse cell types such as fibroblasts, keratinocytes, astrocytes, thymic epithelia, and endothelial cells have been shown to generate IL-1 biological activity (3) that has sometimes been ascribed to a gene product distinct from IL-1α or IL-1β (4, 5). Previously, we had shown that one-third of 27 cell lines cultured from late stage primary and metastatic melanomas secreted an IL-1 biological activity, as detected using the mouse thymocyte costimulation assay (6). In each of three cell lines further tested, this activity was attributed to IL-1α. Northern blot analysis of transcripts from secreting cell lines revealed RNAs 2.2 and 1.6 kilobases long that hybridized to IL-1α and IL-1β probes, respectively. Although these RNAs were of lengths corresponding to authentic IL-1α and IL-1β, the intensity of hybridization was weak (6). The melanoma cell lines contain an IL-1 biological activity that fractionates with an apparent molecular weight of 22,500–27,500, which is significantly higher than that of secreted monocyte IL-1α which has a molecular weight of 18,000 (6). These data made it compelling to determine whether melanoma IL-1 activity is identical to monocyte IL-1 activity or whether it results from a related, but distinct cytokine. Here, we analyze cDNA clones from a metastatic melanoma cell line WM1158 which expresses IL-1 biological activity. Sequence data confirm that this cell line transcribes IL-1α and β genes identical to those in monocytes. In addition, the WM1158 cell line produces a second IL-1α in which the normal isoleucine at codon 145 is replaced by threonine.

Materials and Methods

Construction of the WM1158 melanoma cell cDNA library in the λgt11 vector was described previously (7). A total of 75,000 insert-containing plaques were screened with 32P-labeled DNA probes corresponding to monocyte IL-1α and IL-1β. The IL-1α probe was a 460-base pair insert from pΔ3IL-1α (8) representing 64 base pairs of the 5′ noncoding region and 396 base pairs of the NH2-terminal coding region. The IL-1β probe was a 530-base pair insert from pΔ11IL-1β (9) representing 113 base pairs of the 5′ noncoding region and 417 base pairs of the NH2-terminal coding region. DNA sequence analysis was performed only on positive plaques derived from the tertiary screen.

Recombinant phage were amplified by a miniplate lysate procedure using a LambdaSorb (Promega, Madison, WI) column (10). DNA was isolated from purified phage as described previously (11). Since the cDNA insert from the IL-1β hybridizing plaque contained artificial EcoRI ends, the insert was removed by EcoRI digestion and subcloned into Bluescript plasmid (Stratagene, La Jolla, CA). The insert was sequenced from double stranded DNA using [32P]dATP label (12). The cDNAs from the IL-1α hybridizing plaques could not be removed as intact inserts from the recombinant phage by EcoRI digestion since they contained an internal EcoRI site; in the cDNA cloning procedure, EcoRI sites were protected by in vitro methylation (7). Therefore, these inserts were sequenced directly from the recombinant λ phage by the method of Del Sal et al. (13), using oligonucleotide primers to obtain overlapping sequences on each strand. For all recombinants examined in this study, the nucleotide sequence was obtained from analysis of both DNA strands.

Results and Discussion

We have demonstrated previously that the metastatic melanoma cell line WM1158 expresses IL-1 biological activity as measured in the mouse thymocyte costimulation assay (6). In this study, to determine whether melanoma IL-1 activity represents products of the same genes as monocyte IL-1, we screened a λgt11 cDNA library of WM1158 (7) with 32P-labeled DNA probes for monocyte IL-1α (8) and IL-1β (9). Positive plaques obtained from the tertiary screen of the melanoma cDNA library were used for sequence analysis.

The longest cDNA insert from one of the IL-1β hybridizing plaques was subcloned into Bluescript plasmid and sequenced. It was found to contain a complete open reading frame identical to monocyte IL-1β, with a glutamic acid encoded at the sixth codon (Fig. 1). The sixth codon of monocyte IL-1β may encode either glutamic acid or lysine (9, 14–16).

The cDNA inserts from several of the IL-1α hybridizing plaques from the WM1158 library were sequenced directly from recombinant λ phage template DNA. The sequences of four independent cDNA clones, referred to as IL-1α1, were the same.
as monocyte IL-1α (8, 16, 17) with the following exceptions: (a) they encoded a threonine at position 145 rather than isoleucine; and (b) they had a deletion of 4 base pairs (ATTTC) in the 3′ noncoding region which in monocytes can be either glutamic acid or lysine. The DNA sequence of the melanoma IL-1α is identical to monocyte IL-1α.

The DNA sequence of the melanoma IL-1α is identical to the first nucleotide of the AUG initiation codon. The position of the most amino-terminal codon and the last codon present in each cDNA is indicated; in all cases they correspond to monocyte IL-1α and IL-1β. The DNA sequence of melanoma and monocyte IL-1β are identical; the sixth codon of melanoma is glutamic acid, which in monocytes can be either glutamic acid or lysine. The DNA sequence of the melanoma IL-1α is identical to monocyte IL-1α except that threonine occurs at position 145 rather than isoleucine and there is a four base-pair deletion (ATTTC, nucleotides 1744–1747, represented by the black bar) in the 3′ noncoding region. The DNA sequence of the melanoma IL-1α is identical to monocyte IL-1α.

These data indicate that the IL-1 detected in the conditioned medium of melanoma cell line WM1158 is a product of the same IL-1α and -β genes as in monocytes. The significance of the sequence differences between monocyte IL-1α and melanoma IL-1α is not apparent. It is unlikely that they represent errors due to sequencing or incorporation mistakes produced by the reverse transcriptase since the same substitution was obtained with 4 independent clones. It is possible that these differences could reflect mutational changes in the melanoma cells. If this were true, then the melanoma cell line could contain both the normal IL-1α allele and a mutated IL-1α allele containing an amino acid replacement at position 145 and a deletion in the 3′ untranslated region within the gene. Alternatively, these variations may represent allelic differences of no particular importance, similar to the variations seen in codon 6 of monocyte IL-1β (9, 14–16).

From this study, it is clear that monocytes and WM1158 metastatic melanoma cells transcribe the same IL-1α and -β genes, respectively, and that the IL-1 biological activity produced in melanoma cells is most likely due to the action of IL-1α and -β. Unlike monocytes, the IL-1 activity secreted by melanoma cells appears to be primarily IL-1α rather than IL-1β (6). The molecular weight of secreted IL-1 activity of melanoma cells exceeds that produced by monocytes (6), which suggests a difference in posttranslational modification. For example, in melanoma cells, the respective forms of precursor IL-1α and -β may be cleaved at sites which are not used in monocytes. Identification of the melanoma gene products associated with IL-1 biological activity as bona fide IL-1α and -β should make it possible to explore the function of endogenously produced IL-1 in tumor progression.

References

J. L. Bennicielli and D. Guerry. Characterization of interleukin 1 production by human melanoma cell lines, unpublished results.
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