# Integrative genomic analyses on interferon-λs and their roles in cancer prediction

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**Abstract.** Interferon (IFN)-λs, including IFN-λ1, IFN-λ2, and IFN-λ3, are a newly described group of cytokines distantly related to the type I IFNs and IL-10 family members. Besides the antiviral activity, IFN-λs were reported to inhibit various tumor growths *in vitro* and *in vivo*. Herein, we identified IFN-λ genes from the genome sequences of the human, chimpanzee, macaque, orangutan, mouse, rat and dog, and found that the locations and copy of a specific IFN-λ varied in different genomes, not just the copy of IFN-λs. We found human IFN-λs were expressed in fetal retina, fetal brain and T cells by ESTs search. Moreover, IFN-λs were also found to express in bladder cancer, blood cancer, breast cancer, glioma, head and neck cancer and lung cancer tissues. Three tumor-related transcriptional factors (steroidogenic factor-1, Wilms tumor 1 and P53) binding sites were identified within the 1.0-kb regions upstream of the transcriptional start site of human IFN-λs. Meta-analysis of the prognostic value of IFN-λ genes in various cancers showed that the expression of IFN-λs are indeed related to the cancer prognosis in certain types of cancer. It can be predicted that IFN-λs take part in the cancer development by the regulation of expression of IFN-λs related to the SF-1, P53 and WT-1.

## Introduction

Interferon (IFN)-λs including IFN-λ1, IFN-λ2, and IFN-λ3, also known as IL-29, IL-28A, or IL-28B, are a newly described group of cytokines distantly related to the type I IFNs and IL-10 family members. Sheppard *et al* (1) mapped the IFN-λ1, IFN-λ2, and IFN-λ3 genes in chromosome 19q13.13. The gene localization appeared distinct from that of the related type I IFNs, which are co-localized on chromosome 9. Similar to the IL-10 gene family, IFN-λs contain multiple exons, IFN-λ2 and IFN-λ3 have six, and IFN-λ1 has five exons. In contrast, type I IFNs are encoded within a single exon. The deduced IFN-λ1 protein contains 200 amino acids, which is 81% identical to IFN-λ2 and IFN-λ3, but it shares only low homology with IL-10, IFN-A2, and IL-22. IFN-λs have a conserved cysteine pattern and amphipathic profile similar to other helical cytokine family members.

It is well known that IFN-λs have antiviral activity against a broad spectrum of viruses including human immunodeficiency virus type 1 (HIV-1) (2), influenza A (3-4), Apeu virus (5), hepatitis C virus (HCV) (6-7), hepatitis B virus (HBV) (8), respiratory syncytial virus (RSV) (9), encephalomyocarditis virus (EMCV) (10) and West Nile virus (11). Another important function of IFN-λs is their potential antiproliferative activities. IFN-λs were reported to inhibit proliferation of human glioblastoma LN319 cell line (12), human neuroendocrine BON1 tumor cells (13), human keratinocyte cell line HaCaT (14), human fibrosarcoma 2fTGH cell line (15) and murine BW5147 thymoma cell line (15) *in vitro*.

Fox *et al* used gene structure analysis and comparative genomics to identify IFN-λs from other mammalian genome sequences and found that there is one to potentially nine copies of IFN-λ genes in each genome (16). IFN-λs can be induced in various cell lines and primary cells by dsRNA or viral infection (10,17,18). It is hypothesized that IFN-λs and type I IFN genes are regulated by a common mechanism. The IFN-λ1 is regulated by virus-activated IFN regulatory factor (IRF) 3 and IRF7, resembling the regulation of the IFN-ß. IFN-λ2/3 expression is mainly controlled by IRF7, resembling the regulation of IFN-α (19).

In the present study, we identified IFN-λ genes from the genome sequences of the human, chimpanzee, macaque, orangutan, mouse, rat and dog by comparative genomic analyses. Conserved transcription factor-binding sites within promoter regions of human IFN-λ genes were then searched. Furthermore, meta-analysis of the prognostic value of IFN-λ genes in various cancers was also performed.

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## Materials and methods

*Identification of novel IFN-λs in mammals and comparative genomic analyses*. IFN-λ genes were searched in the genome sequences of human *(Homo sapiens)*, chimpanzee *(Pan troglodytes)*, macaque *(Macaca mulatta)*, orangutan *(Pongo pygmaeus)*, mouse *(Mus musculus)*, horse, rat *(Rattus norvegicus)* and dog *(Canis familiaris)* using human and mouse IFN-λs as queries. The assemblies used were human NCBI 36, chimpanzee CHIMP2.1, macaque MMUL 1.0, orangutan PPYG2, mouse NCBI m37, rat RGSC 3.4 and dog Canfam 2.0. The identified putative IFN-λ genes were BLASTed against the nr database of GenBank to confirm that the best hits were IFN-λ genes. Conserved transcription factor-binding sites within promoter regions of human IFN-λ genes, IL-10 and IFN-γ were then searched by using the Patch program (http://www.gene-regulation.com) as well as manual inspection as previously described (20-31).

*Comparative proteomic analyses*. Amino-acid sequences of the identified IFN-λ genes were used for the phylogenetic analysis. The phylogenetic tree of IFN-λ genes were obtained by using ML (maximum likelihood) (PHYML v2.4.4) (32) and NJ (neighbor-joining) (MEGA 3.0) (33) methods, and the reliability was evaluated by the bootstrap method with 1,000 replications.

*In silico expression analyses*. Expressed sequence tags (ESTs) derived from human IFN-λs, IL-10 and IFN-γ were searched by using the BLAST programs as previously described (21-31). Human IFN-λ1 (NM_172140), IFN-λ2 (NP_742150), IFN-λ3 (NM_172139), IL-10 (NM_000572) and IFN-γ (NM_000619) were used as query sequences for the BLAST programs.

*Meta-analysis of the prognostic value of IFN-λ, IL-10 and IFN-γ genes in cancer*. A database named 'PrognoScan' has been developed recently (34), which contains a large collection of publicly available cancer microarray datasets with clinical annotation. When a gene is given, PrognoScan displays a summary of tests in a table format for the gene. The parameters include dataset, cancer type, subtype, endpoint, cohort, contributor, array type, probe ID, number of patient, optimal cutpoint, *P*min and *P*cor. It can also be used as a tool for assessing the biological relationship between gene expression and cancer prognosis. PrognoScan employs the minimum P-value approach for grouping patients for survival analysis. It provides a powerful platform for evaluating potential tumor markers and therapeutic targets, and is accessible at http://gibk21.bse.kyutech.ac.jp/PrognoScan/index.html. Human IFN-λ, IL-10 and IFN-γ genes were inputted as queries and the data were collected for further analysis.

## Results

*Identification of IFN-λs in mammal genomes*. IFN-λ genes were searched in the genome sequences of human, chimpanzee, macaque, orangutan, mouse, rat and dog. While three IFN-λ genes were identified from human, dog, orangutan genomes, only two IFN-λ genes were identified in macaque and mouse genomes. Interestingly, rat has only one IFN-λ gene in its genome, whereas horse possesses 4 IFN-λ genes in its genome. For chimpanzee genome, three IFN-λ genes were identified, but only one complete IFN-λ sequence was obtained. The other two sequences were not available due to the uncompleted genome sequencing project. The amino acid sequences of the identified IFN-λs can be obtained upon request.

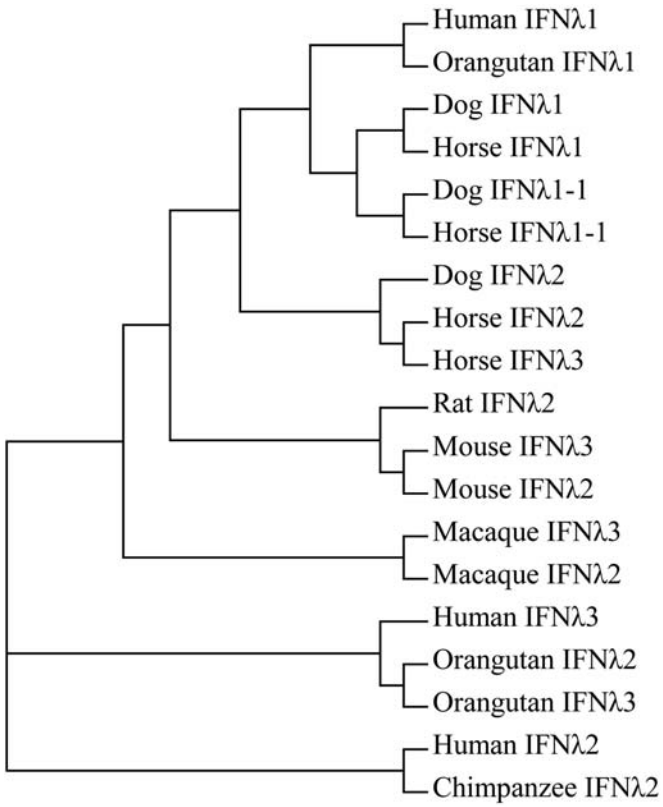


Figure 1. Phylogenetic analysis of IFN-λs. The phylogenetic tree of IFN-λ gene was obtained by using maximum likelihood and neighbor-joining methods. It seemed that IFN-λ distinctly separated from IFN-λ2 and IFN-λ3.

*Comparative proteomics on mammalian IFN-λ genes*. Refined phylogenetic tree using the identified IFN-λ amino acid sequences by ML and NJ methods was almost identical. IFN-λ1s were clustered into one group and distinctly separated from IFN-λ2 and IFN-λ3 (Fig. 1). However, IFN-λ2 and IFN-λ3 were clustered into another group and not separated from each other.

*Expression profile of human IFN-λs*. Human IFN-λ1 was expressed in fetal brain and T cells whereas human IFN-λ2 and -λ3 were expressed in fetal retina, brain and T cells. When searched in PrognoScan database, human IFN-λs were also found to be highly expressed in bladder cancer, blood cancer, breast cancer, glioma, head and neck cancer and lung cancer tissues. IL-10 was expressed in blood, spleen, lung con-nective tissue and T cells. IFN-λ was expressed in blood, kidney, lymph node and fetal brain.

*Comparative genomics on human IFN-λs, IL-10 and IFN-γ*. Transcription factor-binding sites within the 5'-region of human IFN-λs were identified. Within the 1.0-kb regions

Table I. Meta-analysis of the prognostic value of IFN-λs, IL-10 and IFN-γ genes in cancer.

| Genes | Cancers | | | | | |
|---|---|---|---|---|---|---|
| | Bladder | Blood | Breast | Glioma | Head and neck | Lung |
| IFN-λ1 | 0/1 | 0/2 | 2/4 | 0/1 | 0/1 | 0/2 |
| IFN-λ2 | 0/1 | 0/2 | 1/4 | 0/1 | 0/1 | 0/2 |
| IFN-λ3 | 1/1 | 1/2 | 1/4 | 0/1 | 0/3 | 0/2 |
| IFN-γ | 0/2 | 0/7 | 2/29 | 0/2 | 0/1 | 0/8 |
| IL-10 | 0/2 | 0/7 | 3/25 | 0/2 | 0/1 | 0/6 |

Data were expressed as n/m. n represents the test number that showed an association between microarray expression of genes and cancer prognosis with 5% significance level. m represents the test number.

upstream of the transcriptional start sites, four steroidogenic factor-1 (SF-1), three Wilms tumor 1 (WT1) and one P53 binding sites were identified for human IFN-λ1; three SF-1, four WT-1 and two P53 binding sites were identified for human IFN-λ2; five WT-1 and two P53 binding sites was identified for human IFN-λ3. Two SF-1, WT-1 and P53 binding sites were identified for human IL-10. Only one SF-1 and WT-1 and P53 binding sites were identified for human IFN-γ (Fig. 2)

*Meta-analysis of the prognostic value of IFN-λ, IFN-γ and IL-10 genes in cancer*. Using the databases detecting the expression of IFN-λs, 2 out of 11 tests showed an association between microarray expression of IFN-λ1 and cancer prognosis with 5% significance level. By clicking the probe ID of these positive breast cancer cases in the list, we found the Rotterdam cohort for distant metastasis-free survival (DMFS) and recurrence-free survival (RFS) that patients can be dichotomized at the 10 percentile to give the minimum P-value and the group with low IFN-λ1 expression has poorer survival chance (*P*cor=0.002) (Table I). Moreover, these two cases showed no association between expression in IFN-λ2 and IFN-λ3 with cancer prognosis. One out of 11 tests showed an association between microarray expression in IFN-λ2 and cancer prognosis with 5% significance level. Rotterdam cohort for RFS that patients can be dichotomized at the 84 percentile to give the minimum P-value and the group with high IFN-λ2 expression has poorer survival chance (*P*cor=0.0087) in this breast cancer case (Table I). Three out of 13 tests showed an association between microarray expression in IFN-λ3 and cancer prognosis with 5% significance level. In the bladder cancer case, Rotterdam cohort for overall survival (OS) that patients can be dichotomized at the 33 percentile to give the minimum P-value and the group with high IFN-λ3 expression has poorer survival chance (*P*cor=0.0366).

In the blood cancer (multiple myeloma) case, Rotterdam cohort for Arkansas that patients can be dichotomized at the 20 percentile to give the minimum P-value and the group with low IFN-λ3 expression has poorer survival chance (*P*cor=0.0071). In the breast cancer case, Rotterdam cohort for RFS that patients can be dichotomized at the 88 percentile to give the minimum P-value and the group with high IFN-λ3 expression has poorer survival chance (*P*cor=0.0066), very similar to the result of IFN-λ2 (Table I). Using the databases detecting the expression of IFN-γ and IL-10, 2 out of 49 tests and 3 out of 43 tests showed an association between microarray expression of IFN-γ and IL-10 with cancer prognosis, respectively (Table I).

## Discussion

By comparative genomic analysis, we found that IFN-λ1, IFN-λ2 and IFN-λ3 genes exist in two primate (human and orangutan) genomes. However, only IFN-λ2, and IFN-λ3 genes were found in the other primate (macaque) genome. In dog genome, two IFN-λ1s are clustered in the chromosome 1, whereas the IFN-λ2 is located in chromosome 24. However, two IFN-λ1s are identified in chromosomes 10 and 22 in horse genome, whereas IFN-λ2 and IFN-λ3 genes are found only in chromosomes 10. It seemed that the locations and copy of specific IFN-λs varied in different genomes, not just the copy of IFN-λs. It may be due to the gene conversion and gene duplication, which have shaped the evolution of the IFN-λ gene family in eutherian species. It is similar to the evolution of IFN-α, which consists of a 13 member multigene family in human genome (35). Members of the IFN-α gene family in different species normally reside in close proximity within a single chromosome. Phylogenetically, IFN-α family members in eutherians (placental mammals) cluster together in a species-specific manner except for closely related species (i.e. *Homo sapiens* and *Pan troglodytes*) and formed due to both gene conversion and gene duplication (36).

IFN-λ expression was detected in human blood, brain, lung, ovary, pancreas, pituitary, placenta, prostate, and testis (1). They are also induced in various cell lines and primary cells by dsRNA or viral infection (1,14). In the present study, we found human IFN-λs were also expressed in fetal retina, fetal brain and T cells by ESTs search, indicating that IFN-λs are early products of human life, which most likely participate in the innate immunity in early life. Other type I IFNs (IFN-γ) and IL-10 family members (IL-10) showed different expression patterns with IFN-λs. Moreover, IFN-λs were also found to express in bladder cancer, blood cancer, breast cancer, glioma, head and neck cancer and lung cancer tissues. IFN-λ and IL-10 were also found to express in these cancers.

Former study found that IFN-λ1 and IFN-λ3 gene promoters had functional IFN-stimulated response element and NF-kappaB binding sites and that the IFN-λ1 gene is regulated by virus-activated IRF3 and IRF7, whereas IFN-λ2 and IFN-λ3 gene expression is mainly controlled by IRF7 (19). In the present study, we identified different numbers of SF-1, WT1 and P53 binding sites located within the 1.0-kb regions upstream of the transcriptional start site of human IFN-λ1, 1234IFN-λ2 and IFN-λ3 genes, though the amino acid sequences of human IFN-λ2 and IFN-λ3 showed high similarity. The IFN-λ1 and IFN-λ3 gene promoters were different at least in the SF-1 binding sites. It suggested that

TTAACCAGTCAAGGTGACACCTAAAATTAACCATCACAATTATAAAAATAACTACTCAGAGAAACATTAGGA
GCATGAACTGAAATTAGTTAATGGGACATTCTTAAACCAATGGCAGAAGCTCCTTCTTGGCCAGGAGCAGTG
GCTCATGCCTTTAATACTAGCACTTTGCGAGGCTGAAGCAGGAGGATGGCTTAAGGCCAGGAGTTCAAGACT
GGCCTGGGCAACATAGTGAGACCCCTATCTCTACAAAAATAAATAAATAAATAATAAAGTAAGGTGGTGGCT
CACGCCTGTAATCCCAGCACTTTGGGAGGCCAAGGCAGGCAGATCATCTGAAGTCAGGAGTTCGAAGCCAGC
GTGACCAACATAGTAAAACCCAGTCTCTACTAAAAATACAAAAACTAGCCAGGCGTGATGGCATGCACCTGT
AATCCCAACTACTTAGGAGGCTGAGGCAGGAGAATCGCTTCAACTCGGGAGGCAGAAGTTGCAGTGAGCCAA
GATTGCACCATTGCACTCCAGCCTGGGCAACAAGAGCAAAACTACGTCTCAAAAAATAATAATAACAATAAA
ATAAAAAACAAGCTTTTTTTTTTTTGAAACAGGATCTCACTCCATCACCCAGGCTGGAGTGCAGTGGCACGA
TCTTGGCTCACTGCAACCTCCGCCTCCCGGGTTCAAGTGATTCTCATGCCTCGGCCTCCTGAGTAGCTGAGA
CCACAGGCGCATGCCACCACACCTGGCTAATTTAGAATAAAAAAGAAGCTTCCTCTCTGCCACTCAGGTAGC
CTTATCCCTAATCTCAGCCTCCGTCAGGGACTCCCTGAGGCCAGTTGGCTGAAAGCTGCCCAGGGAGTTCTA
AGGATTTCAGTTTCTCTTTCCTTCTTGATGCAGCTCCCAGCTCACTTGGCCCTGCCCACACCTGTTCCCTCA
TCAGGCTCCCAGACGGGCCCCGCCCACTCATGCCTCTTAAGTCAAAGTGGAAATTCTCATTTCCAATTACCT
TTTCACTTTACACACATCATCTTGGATTGCCCATTTTGCGTGGCTAAAAAGCAGAGCCATGCCGCTGGGGAA
GCAGTTGCGATTTAGCC**ATG**

B. IFN-λ2

TGGGCGTGGTGGTGCATGCCTATAGTCCTTTCTACTCGGGAGGCTGAAGCAGAAGAATCCCTTGAACCAAGG
AGGTGGAGGTTGCAGTGAGTCGAGATCACGCTGCTGCACTCCAGCCTGGGCTACAGAGCAAGACTCCATCTC
AAAAAAAAAAGAAAAAAGAAAGAAAGAAAGAAATCATGGCCTCTGGGCACAGTGGCTCATGCCTGCAACCCC
AGCAATTTGGGAGGCCAAGACAGACAGATCACTTGACGTCAAGAGTTCGAGACCAGCCTGGCCAATTGGTGA
ACTGTCATCTCTACTAAAACCATAAAAATTAGCTGGGAATGGTGGCACAAATCTGTAATCTCAGCTACTTGG
GAGGCTAAGGCAAGAGAATCGCTTGAACCCAGGAGGTGGAGGTTGCAGTTAGTCAAGATTTTGCACTGCACT
CCAGCCTGGGTGACCGAACAAGACCCTGTCTCAAAATATATATATATATATGCCAGGAGGGGTGGCTCAG
GCCTGTAATCTCAGCACTTTAATAGGCTGGGTGAGGAGGATGGCTTGAGCCCAGGAGTTTGAGGCTGCAGTG
AGCTGTGATCATGCCATTGCACTCCAGTGACAGAGTGAGACCCTGTCTTAAACAACAACAAAACCAGAGCAG
GTGGAATCCTCCTGGGAACATACCTTCCTGTAGGTTACCCCTGAGTCTCCATCAGTTTCTCTTTCCCTCCAG
CTGCTCATCTGGCTCACTAGCCCTGCCCTGCTCTGGGCTTTCCCAGCCTGGGGCTCCCCTGGTGGCCGGTGT
CTTACCTGAGGCTGTGTTTTCACTTTTCCTACATCAGCTGGGACTGCCCTTCTGTCAGGGATAAAAGCTGCC
CCATGGAGCTCAGGCAGGAATTACATCCCAGACAGAGCTCAAAACTGACAGAAAGAGTCAAAGCCAGGACAC
AGTCTGAGATCCAGAAGAGGGGACTGAAAAGAACAGAGACTCCAGACAAGACCCAAACAGACCCTGGGTGAC
AGCCTCAGAGTGTTTCTTCTGCTGACAAAGACCAGAGATCAGGA**ATG**

C. IFN-λ3

TTTGAACCCCAGACCCTTCATCTCCAAGACCCATGCTCTTCACCACTGCCTGAACTTCCCTAAGAAAGACGG
CACCCACGTGGTGTCCTTCAAGTCCTTCGTCACACCTCAATTCTTGAGCAGAGCCTCATATTCCTGAGTCCT
TCCTTGCCTGGGCAATTAAGAAATATTGGCCTCTGGGCATGGTGGCTCACACTGAAATCCCAGCAATTTGGG
AGGCCTAGACAGAGAGATGACTTGACATCAGGAATTTGAGACCAGCCTTGCCAACATGGTGAAACGCCATCT
CTACTAAAAATATAAAAATTAGCTGGGAATGGTGGCACAAATCTGTAATCTCAGCTACTTGGGAGGCTAAGG
CAAGAGAATTGCTTGAACCCAGGAGGCGGAGGTTGCAGTTAGCCAAGATTTTGCACTGCACTCCAGCCTGGG
TGACCGAACAAGACCCTGTCTCAAAATATATATATATATATATATATATATGCCAGGAGTGGTGGCTCAG
GCCTGTAATCTCAGCACTTTAATAGGCTGGGTGAGGAGGATGGCTTGAGCCCAGGAGTTTGAGGCTGCAGTG
AGCTGTGATCATGCCATTGCACTGCAGTGACAGAGTGAGACCCTGTCTTAAACAACAACAAAACCAGAGCAG
GTGGAATCCTCTTGGGAACATACCTTCCTGTAGGTTACCCCTGAGTCTCCATCAGTTTCTCTTTCCCTCCAG
CTGCTCATCTGGCTCACTAGCCCTGCCCTGCTCTGGGCTTTCCCAGCCTGGGGCTCCCCTGGTGGCCGGTGT
CTTACCTGAGGCTGTGTTTTCACTTTTCCTACATCAGCTGGGACTGCCCTTCTGTCAGGGATAAAAGCTGCC
CCATGGAGCTCAGGCAGGAATTACATCCCAGACAGAGCTCAAAACTGACAGAAAGAGTCAAAGCCAGGACAC
AGTCTGAGATCCAGAAGAGGGGACTGAAAAGAACAGAGACTCCAGACAAGACCCAAACAGACCCTGGGTGAC
AGCCTCAGAGTGTTTCTTCTGCTGACAAAGACCAGAGATCAGGAATGAAACTAGGTGAGTCCCACATCTCTG
TCCGTGCTCAGCTCCTGCAGCCCCTGCCCTCAGTGGGCAGCCTCTGCATTCCCTCAGCTCCCTTTCTCTCTG
TGACACAGAC**ATG**

Figure 2. The identification of transcription factor-binding sites within the 5'-region of human IFN-λ1 (A), IFN-λ2 (B) , IFN-λ3 (C).

human IFN-λ2 and IFN-λ3 play different roles in their function due to different expression models. Moreover, IFN-λs showed different promoter structures with IFN-γ and IL-10.

WT1 is a zinc finger DNA-binding protein and acts as a transcriptional activator or repressor depending on the cellular or chromosomal context. The WT1 gene is overexpressed in leukemias and various types of solid tumors, and the WT1 protein was demonstrated to be an attractive target antigen for immunotherapy against these malignancies (37). The p53 gene is mutated in about half of all human tumors. p53 is a

D. IL-10

TTATTTCAACTTCTTCCACCCCATCTTTTAAACTTTAGACTCCAGCCACAGAAGCTTACAACTAAAAGA
AACTCTAAGGCCAATTTAATCCAAGGTTTCATTCTATGTGCTGGAGATGGTGTACAGTAGGGTGAGGAA
ACCAAATTCTCAGTTGGCACTGGTGTACCCTTGTACAGGTGATGTAATATCTCTGTGCCTCAGTTTGCT
CACTATAAAATAGAGACGGTAGGGGTCATGGTGAGCACTACCTGACTAGCATATAAGAAGCTTTCAGCA
AGTGCAGACTACTCTTACCCACTTCCCCCAAGCACAGTTGGGGTGGGGGACAGCTGAAGAGGTGGAAAC
ATGTGCCTGAGAATCCTAATGAAATCGGGGTAAAGGAGCCTGGAACACATCCTGTGACCCCGCCTGTAC
TGTAGGAAGCCAGTCTCTGGAAAGTAAAATGGAAGGGCTGCTTGGGAACTTTGAGGATATTTAGCCCAC
CCCCTCATTTTTACTTGGGGAAACTAAGGCCCAGAGACCTAAGGTGACTGCCTAAGTTAGCAAGGAGAA
GTCTTGGGTATTCATCCCAGGTTGGGGGGACCCAATTATTTCTCAATCCCATTGTATTCTGGAATGGGC
AATTTGTCCACGTCACTGTGACCTAGGAACACGCGAATGAGAACCCACAGCTGAGGGCCTCTGCGCACA
GAACAGCTGTTCTCCCCAGGAAATCAACTTTTTTTAATTGAGAAGCTAAAAAATTATTCTAAGAGAGGT
AGCCCATCCTAAAAATAGCTGTAATGCAGAAGTTCATGTTCAACCAATCATTTTTGCTTACGATGCAAA
AATTGAAAACTAAGTTTATTAGAGAGGTTAGAGAAGGAGGAGCTCTAAGCAGAAAAAATCCTGTGCCGG
GAAACCTTGATTGTGGCTTTTTAATGAATGAAGAGGCCTCCCTGAGCTTACAATATAAAAGGGGGACAG
AGAGGTGAAGGTCTACACATCAGGGGCTTGCTCTTGCAAAACCAAACCACAAGACAGACTTGCAAAAGA
AGGC**ATG**

E. IFN-γ

CCACCTTCTGGGTTCAAGCCATTCTCCTGCCTCAGCCTCCCTAGTAGCTGAGATTACAGGCATACACCA
CCATGCCTGGCTAATTTTTTTGTATTTTTAGTAGAGATGGGGTTTCACCATATTGGCTAGGCTGGTCTC
AAACTCCTGACCTTGTGATCCACCCGCCTCAACCTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACTG
CGTCTGGAACTCCCCCTGGGAATATTCTCTACACTGTATTTCAAGGATTTAATATGACAAAAAGAATGT
CAAATACCTTATTAACAATGTAGTATATTGATGCATACTGAAGTACTATTTGGGATATATTGGTTTAAA
TACAATATATTTTAAAATTATATTTACCTTTTAAAAAAACTTTTATTAATGAGGCTACTAGATCATTTA
AATTTACCTGTGTGGCTTGTATTGTATTTCTACTGGGCAGTGCTGATCTAGAGCAATTTGAAACTTGTG
GTAGATATTTTACTAACCAACTCTGATGAAGGACTTCCTCACCAAATTGTTCTTTTAACCGCATTCTTT
CCTTGCTTTCTGGTCATTTGCAAGAAAAATTTTAAAAGGCTGCCCCTTTGTAAAGGTTTGAGAGGCCCT
AGAATTTCGTTTTTCACTTGTTCCCAACCACAAGCAAATGATCAATGTGCTTTGTGAATGAAGAGTCAA
CATTTTACCAGGGCGAAGTGGGGAGGTACAAAAAAATTTCCAGTCCTTGAATGGTGTGAAGTAAAAGTG
CCTTCAAAGAATCCCACCAGAATGGCACAGGTGGGCATAATGGGTCTGTCTCATCGTCAAAGGACCCAA
GGAGTCTAAAGGAAACTCTAACTACAACACCCAAATGCCACAAAACCTTAGTTATTAATACAAACTATC
ATCCCTGCCTATCTGTCACCATCTCATCTTAAAAAACTTGTGAAAATACGTAATCCTCAGGAGACTTCA
ATTAGGTATAAATACCAGCAGCCAGAGGAGGTGCAGCACATTGTTCTGATCATCTGAAGATCAGCTATT
AGAAGAGAAAGATCAGTTAAGTCCTTTGGACCTGATCAGCTTGATACAAGAACTACTGATTTCAACTTC
TTTGGCTTAATTCTCTCGGAAACG**ATG**

Figure 2. The identification of transcription factor-binding sites within the 5'-region of human IL-10 (D) and IFN-γ (E). SF-1-binding sites (underline), WT-1-binding sites (double underline), and P53-binding sites (dotted line) are indicated. The transcriptional start sites (ATG) of human IFN-λs, IL-10 and IFN-γ are indicated in bold.

transcription factor whose activity gives rise to a variety of cellular outcomes, most notably cell cycle arrest and apoptosis, eliminating cancer-prone cells from the replicative pool (38). SF-1 is an orphan nuclear receptor that regulates the transcription of an array of genes involved in reproduction, steroidogenesis, and male sexual differentiation. SF-1 amplification and overexpression are found in various tumors (39-41). IFN-λs were found to inhibit tumor growth *in vitro* and *in vivo* (12-15). However, the detailed role in the development of cancer was still unknown.

Meta-analysis of the prognostic value of IFN-λ genes in various cancers found that the expression of IFN-λ is indeed related to the cancer prognosis in certain types of cancer. Moreover, the expression of human IFN-λ2 and IFN-λ3 was differently related to the cancer prognosis. The expression of IFN-λs related to the cancer prognosis is different from other type I IFNs (IFN-γ) and IL-10 family members (IL-10). By combining the identifications of transcription factor-binding sites related to tumors in the promoter regions of IFN-λs, it can be predicted that IFN-λs take part in cancer development by the the regulation of expression of IFN-λs related to the SF-1, P53 and WT-1.

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